DISS. ETH NO 25801

Local sleep-like events during wakefulness and long term space travel

A thesis submitted to attain the degree of
DOCTOR OF SCIENCES of ETH ZURICH
(Dr. sc. ETH Zurich)

presented by

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2019

Abstract

Since the Moon race in the 60s, human kind has continuously been orbiting Earth. However, before we can envision a permanent settlement on the Moon and explore our solar system, human physiology adaptation challenges need to be answered.

Adequate sleep quantity and quality are required for good cognitive performances and chronic sleep restriction comes with a high cost. Sleep quality and sleep quantity are regulated by two interacting processes, the homeostatic and the circadian process. Electroencephalographic (EEG) theta activity (5-7Hz) during wakefulness reflects both the homeostatic and the circadian process. The circadian influence can be seen by a diurnal oscillation of theta activity. The homeostatic component, on the other hand, builds up with the time awake. Theta activity is therefore considered a sleep pressure marker and was associated with alertness and subjective daytime sleepiness. Intracranial recordings in rats showed that an increase of sleep pressure was also linked to neuronal "off" periods during wakefulness, similar to what can be recorded during slow waves sleep. These local sleep-like events during wakefulness have also been studied in the human EEG as markers of sleep pressure and widespread local sleep-like events are thought to be responsible for the decrease in cognitive performances under high sleep pressure conditions.

Sleep quality and quantity in space will be key for maintaining astronaut's cognitive functions and improving missions' success rate. Even though astronauts are allocated enough time to sleep, a decrease of sleep quantity was reported on the International Space Station (ISS). Furthermore, for proper dissipation of sleep pressure, the quality of sleep is also important. In space, sleep quality might be impacted by external factors such as microgravity, isolation, confinement, circadian misalignment, chronic stress, temperature, light and noise disturbances. Besides space missions, analogue missions are opportunities to study human acclimatisation to space-like environment. Concordia station in Antarctica is one of the most remote human outpost on Earth. Studies conducted in Antarctica suggested that isolation, high altitude and constant darkness might disturb sleep in a similar fashion as on the ISS.

In this thesis we investigated sleep pressure markers on the ISS. Then, to disentangle the effects of space environmental factors, we studied sleep pressure markers during a space analogue mission on Earth. Additionally, we studied sleep pressure's impact on cognitive performances and we explored novel countermeasures to enable human deep space travel. In this regard, we subdivided our work into three lines of research with three associated research papers.

First, we studied sleep pressure markers on ISS astronauts during their mission. Our analyses added evidence of the negative effects of the ISS environment on sleep and provided first evidence for increased sleep pressure in space. We observed a global increase of theta power in space and we showed that local sleep-like events are more widespread over the scalp

(i.e. increased globality) in space compared to Earth. In addition, we reported an increase of slow reaction times after two months in space and reaction times were correlated with the globality of the local sleep-like events. However, the performances of the astronauts in a visuomotor task were not associated with local sleep-like events

Second, we investigated if long term isolation in a space analogue station impacts sleep pressure in a similar fashion. In our study, unlike on the ISS, we found no differences in theta power during the isolation period at the Concordia Antarctica station. Nonetheless, we found that in the evening, high sleep pressure in the right frontal cortex was linked to psychological strain. In addition, we showed that at noon, physical activity could be envisioned as a countermeasure for high sleep pressure.

Third, we presented hibernation-like state as an enabler for manned missions beyond Earth orbit. Hibernation is a natural solution to cope with severe resource restrictions and hostile environments. We reviewed the current understanding of hibernation mechanisms and we reported hibernation's protective potential as a countermeasure for space environmental factors.

Abstract

Depuis la conquête spatiale des années 60, l'humanité a constamment maintenu une présence en orbite autour de la Terre. Toutefois, avant de pouvoir envisager un village lunaire et continuer à explorer notre système solaire, la physiologie humaine doit faire face à certains défis d'adaptation.

Une qualité et une quantité adéquate de sommeil est nécessaire pour maintenir les fonctions cognitives et le manque de sommeil a un coût important. La qualité et la quantité de sommeil sont régulés par deux processus interagissant entre eux, le processus homéostatique et le processus circadien. L'activité thêta (5-7Hz) enregistrée par électroencéphalographie (EEG) durant l'éveil, reflète aussi bien le processus homéostatique que le processus circadien. Le processus circadien influence l'activité thêta de façon diurne. Le processus homéostatique, quant à lui, augmente avec le temps éveillé. L'activité thêta est ainsi considérée commun marqueur de la pression de sommeil et est associée à l'attention et à la somnolence durant le jour. Chez les rats, des enregistrements intracrâniens ont montré que la pression de sommeil est aussi liée à des périodes "off" durant l'éveil. Périodes "off" similaires à celles observées durant les ondes lentes du sommeil. Ces épisodes locaux, semblables au sommeil, présent durant l'éveil ont également été étudié chez les humains via EEG, comme marqueur de la pression de sommeil. De plus, les épisodes qui s'étendent dans le cortex sont sans doute responsable de la perte de performance cognitive lorsque la pression de sommeil est importante.

La qualité et la quantité du sommeil dans l'espace seront un élément clé du maintien des fonctions cognitives des astronautes et permettra d'améliorer les chances de réussite des missions. Même si les astronautes ont suffisamment de temps pour dormir, une réduction de la quantité de sommeil a été observée dans la Station Spatiale Internationale (ISS). De plus, pour une dissipation optimale de la pression de sommeil, la qualité du sommeil est également importante. Dans l'espace, la qualité du sommeil peut être affectée par des facteurs externes tels que la microgravité, l'isolation, le confinement, un mauvais alignement du rythme circadien, d'un stress chronique, des changements de température ou encore du bruit. Outre les missions spatiales, les missions analogues sont d'excellentes opportunités pour étudier l'acclimatation des humains à un environnement semblable à l'espace. En antarctique, la station de recherche Concordia, est l'un des postes les plus reclus de la terre. Les études qui ont été menées à la station Concordia ont révélées que l'isolation, en plus de l'altitude et de la pénombre permanente, ont de grandes chances de perturber le sommeil, ce d'une façon similaire à ce qui a pu être observé dans l'ISS.

Au sein de cette thèse, nous avons étudié la pression de sommeil dans l'ISS. Puis, pour comprendre les effets des facteurs environnementaux spécifique au spatiale, nous avons étudié la pression de sommeil lors d'une mission spatiale analogue, ici-bas sur terre. De plus, nous avons analysé l'incidence que la pression de sommeil avait sur les performances cogni-

tives et nous avons exploré de nouvelles contremesures qui permettront un jour l'exploration humaine de l'espace lointain. Pour ce faire, nous avons reparti notre travail sur trois axes, représentés par trois articles scientifiques.

Premièrement, nous avons étudié les marqueurs de la pression de sommeil chez les astronautes pendant leur séjour dans l'ISS. Notre analyse à ajouté une preuve supplémentaire des effets négatifs de l'environnement spatiale sur le sommeil et a fourni la première preuve d'une augmentation de la pression de sommeil dans l'espace. Nous avons observé une augmentation globale de l'activité thêta dans l'espace et nous avons montré que les épisodes locaux, semblables au sommeil, sont plus étendus (i.e. augmentation de la globalité) dans l'espace que sur terre. De plus, les temps de réaction sont plus lents deux mois après le début de la mission spatiale et les temps de réaction sont corrélés avec la globalité des épisodes locaux, semblables au sommeil. Cependant, les performances des astronautes lors d'un exercice visuomoteur n'ont aucune association avec les épisodes locaux, semblables au sommeil.

Deuxièmement, nous avons étudié la possibilité que l'isolation long terme dans une station spatiale analogue puisse impacter la pression de sommeil d'une façon similaire. Dans notre étude, contrairement à l'ISS, nous n'avons trouvé aucune différence concernant l'activité thêta, ce tout au long de la période d'isolation en antarctique. Néanmoins, nous avons montré que le soir, une haute pression de sommeil dans la partie droite du cortex frontal, était liée au stress psychologique. De plus, nous avons montré que à midi, l'exercice physique peut être envisagée comme une contremesure contre une pression de sommeil trop élevée.

Troisièmement, nous avons présenté un état semblable à l'hibernation comme un catalyseur pour la conquête spatiale humaine. L'hibernation est une solution naturelle en réponse au manque de ressources et à un environnement hostile. Nous avons revu les connaissances actuelles des différents mécanismes de l'hibernation et nous avons exposé le potentiel des caractéristiques protectrices de l'hibernation, constituant ainsi une contremesure permettant le voyage spatiale.

Citation

To infinity and beyond!
Buzz Lightyear

Contents

Chapter 1

Introduction

1.1 Space physiology and psychology

1.1.1 Space habitats

Manned spaceflights For the last 60 years, ever since Gagarin's Vostok mission in 1961, we have been learning about how the human body adapts in space environment. Since then, human space exploration programmes have been sending humans in Earth's orbit from a few days in the 60's up to a year long in the 90s. It all started with the Vostok, the Friendship, the Gemini, the Voskhod and the Apollo programmes during the 60's Moon race. Followed by the Soyuz programme and the Skylab station in the 70's. In the 80's-90's, the duration of manned spaceflight increased with the space shuttles, the Spacelab and the Mir station. Finally, since 2000, human kind has a permanent outpost in low Earth orbit with the International Space Station (ISS). After the Moon race, the budget allocated to human space exploration decreased considerably and projects like an human settlements on the Moon and on Mars never emerged. Nowadays, with the New Space Wave and the influx of private money into space projects, we can envision once more human settlements beyond low Earth orbit.

Beyond Earth orbit Future space exploration programmes will send humans further and for a longer periods. The roadmap of the agencies consist in exploring the surface of Mars and the Moon. But this time they are planning to stay. Lava tubes are thought to be an ideal place for the installation of an habitat on the Moon and Mars, because of their shielding capacities against meteorites and cosmic radiations. If crews stay longer, they would need to learn how to use the space resources, also called in-situ space resources utilisation (ISRU). With the advent of human settlements on Mars by early 2030 (international exploration roadmap, NASA, SpaceX), the Lunar Orbital Platform-Gateway and the Moon Village plans by 2030, over 30 taikonauts/astronauts/cosmo-

nauts could be on a long-term space exploration mission at the same time. For long duration missions beyond low-Earth orbit health and safety of future space explorers will be crucial [259].

International Space Station Microgravity, vacuum, ionising radiation, extreme temperatures, space debris and micrometeorites constitute the characteristics of space environment. Orbiting at 400km above ground, ISS astronauts are partially protected by Earth's magnetic field, which reduces the dose rate of radiations compared to an interplanetary spaceflight. ISS modules are pressurised with breathable air and temperature is controlled. However, extended microgravity, exposure to radiations and the risk of an impact with a high velocity objects remain (e.g. space debris travelling at 11km/s) [239].

Long term human spaceflight Automated robotic exploration could be more efficient and safer than manned spaceflights. However, decision making, complex tasks, repair and troubleshooting will always require a human presence in space. Moreover, for deep space missions, a communication lag will be induced due to the light speed communication limits (e.g. 20 minutes delay to Mars surface). Hence, rovers can not be operated from Earth, they need to be fully autonomous or operated from a planet orbiter.

The risks of long term manned space travel comprise, 1) physiological changes because of weightlessness and ionising radiations, 2) psychological strain because of isolation, confinement and because crew escape is not possible [259]. Overall, muscle atrophy, bone density loss, upper body fluids shift, intracranial pressure increase, vision impairments, vestibular system impairment, cardiovascular system alterations, arteriosclerosis, oxidative stress, DNA alterations, neurodegeneration, immune system alteration, inflammation, infectious illness, latent virus reactivation, microbiota alteration, sleepiness, behavioural, cognitive and psychiatric disorders have been reported by flight surgeons [40, 238]. For some effects, the human body acclimates to the novel space environment, for others, the symptoms worsen over time in space [263]. However, all along human spaceflight history, only 300 humans have been confronted to space environment, which leaves us with only a small sample size to draw conclusions from. Moreover, space studies are often conducted in uncontrolled experimental conditions.

1.1.2 Space analogue facilities

To overcome space studies' small sample size drawback, ground-based analogues can be exploited to simulate space missions. Space analogue facilities are also used as testbeds for new countermeasures, prior to spaceflight. Each ground based facility mimic another aspect of space environment. Therefore,

some facilities can be more adapted than others for particular countermeasures.

Analogue missions Space agencies (Roscomos, NASA and ESA) have at their disposal a large variety of human space analogue habitats. The most common are analogues that simulate isolation, confinement, light/dark cycles and communication channels far from Earth to analyse the behaviour, the performances and the autonomy of the human crews. The Russian are conducting one year experiments in a Mars transfer vehicle prototype, called MARS 500 and SIRIUS analogue missions. The Hawaiian are running one year long HiSEAS missions in a Mars habitat prototype. The Americans run regularly short missions, for a few weeks, at NASA's aquatic facility, NEEMO, or at their Mars habitat, HERA.

Antarctica There is also about 50 permanent research stations in Antarctica to mimic extreme environments. Wintering in research stations is more comfortable nowadays and the major strain that remain is geographical isolation. Most of Antarctic stations are on the shores. NASA has the biggest station, McMurdo, also on the shores. There is a great difference between a coastal stations and an inland stations. There is only three stations inland, each of them are totally isolated during the winter and at more than 2500m above sea level . The Russian Vostok Station, the European Concordia station and the American Amundsen–Scott South Pole Station have to deal with temperatures as low as -80°C, no sunlight for four months and no access route during the winter. As an example, the European Concordia station is isolated inland during the 8 months of winter with no means to leave the station. This particularity adds an additional stress, since the expedition cannot stop in case of failure. On top of this the station is in high altitude at 3200m, resulting in hypoxia symptoms within the crews.

Bed rest studies To mimic the fluid shift and the hindlimb unloaded paradigm due to microgravity, head-down-tilt bed rest studies are popular. Bed rest studies immobilise participants with the head down at 6° for up to two months. Long term immobilisation lead to changes in skeletal muscles and bone metabolism but also psychological changes can be observed [157]. The German space agency (DLR) recently inaugurated a new bedrest study facility, called Envihab, to conduct two months long analogue missions. Sleep, circadian rhythm, bone and muscle physiology experiments can be conducted there under a highly controlled environment. The primary aim of bed rest studies remain to evaluate microgravity countermeasures effectiveness in analogue missions and to study how countermeasures can be implemented in long term space missions (e.g. physical exercise and artificial gravity) .

Parabolic flights Finally, each single agency organise parabolic flight campaigns, to reproduce short term periods of microgravity. The aircraft gives its occupants a sensation of weightlessness by having its engines compensating for the drag of Earth gravity. To this end, the plane follows a parabolic flight path, to create a 22 seconds weightlessness period at the top of each parabola and 2g periods before and after the vertex.

However, by no means, analogue terrestrial situations could mimic the limited physical activity, nor the chronic stress load endured during a spaceflight, nor could they reproduce the cosmic radiations, nor remove Earth gravitational field.

1.1.3 Microgravity

Before we can think of human deep space exploration, there is hurdles we need to solve. The most documented one are about the lack of gravity. Orbiting Earth in a space station is not representative of a zero gravity environment, but represents a microgravity environment. Astronauts still experience more than 80% of Earth's gravity, however, the free falling effect induced by the high orbiting velocity of the station gives an impression of weightlessness. Interestingly, the weightlessness impacts the human body in many ways.

Muscle and Bone loss Microgravity unloading effect, induce a reduction of bones density and an atrophy of the postural muscles. Bone density decrease from 1 to 2% per month in microgravity, with a high variability between upper body and lower body bones [159]. After a six months on the ISS an overall atrophy of 20% of the postural muscles has been reported [249]. Usually, astronauts need almost two years to entirely recover from their spaceflight and the bone remodelling mechanisms are yet not fully understood [52]. During long term missions in microgravity there is a risk increase of bone fracture, ligaments and tendons alteration and chronic back pain.

Core body temperature Human thermoregulation in microgravity is altered because heat convection cannot be performed by evaporation. A recent study showed an increase of core body temperature by 1°C in space compared to Earth [225]. However, the increase of temperature is more significant after two months on the ISS and takes up to 10 days after landing to return to baseline, which gives reasons to believe that astronauts adapted their temperature setpoint to the new space environments

Vision Most of neuroscience experiments on Skylab, MIR and ISS focused on visual perception, balance and locomotion [68]. In microgravity, the fluid shift toward the upper parts of the body, increase the intracranial pressure

and is thought to damage the optic nerves, leading in extreme cases to optic neuropathy [231].

Vestibular system In microgravity the vestibular system impairment leads to coordination and balance deterioration. The absence of graviceptive cues and the misleading sensory inputs can also lead to short term space motion sickness. Nevertheless the neurovestibular system will adaptation to the novel gravitational environment (i.e. the vestibular system resets after 72 hours) [265, 130] .

Movement coordination The lack of gravity also impacts sensorimotor control, visuomotor coordination and cognitive performance. So far experiments have shown adaptation to the novel space environment [62].

Adaptation, or acclimatisation times vary from a few days for motion sickness (vestibulomotor) to months for visuomotor coordination and vascular adaptation. Nevertheless, the underlying mechanisms allowing weightfulness adaptation are still poorly understood.

1.1.4 Ionising radiations

To enable human deep space travel, the lack of Earth magnetosphere is a major hurdle. In low Earth orbit, ISS astronauts are partially protected by Earth's magnetic field, which reduce the dose of radiation. In an interplanetary spaceflight, this will not be the case anymore. Two forms of radiation are threatening astronauts' health in deep space. There is chronic exposure to galactic cosmic rays and there is short term exposure to solar flares particles [268]. Galactic cosmic rays are ionising, meaning that they strip electrons from atoms around them. In deep space, exposure to low doses of ionising rays is constant. Unfortunately, they are highly penetrating particles and cannot be stopped by the shielding capacities of spacecrafts. On the other hand, occasional solar flares (high energy protons), could be predicted and strategies for emergency sheltering can be developed on board.

For long term human missions outside of low Earth orbit, ionising radiation is a showstopper [98]. The impact on human physiology is a recurrent topic of concern in space meetings. However, because of the complexity of running an experiment showing the direct impact of radiations on humans, this topic is still poorly understood. The maximal lifetime dose equivalent is measured as sieverts (1Sv), a unit of measure, and has been linked to fatal cancer risks [76]. We expect a trip to Mars to exceed by 10 times the maximal yearly dose of exposure recommended for a human being. The round trip would expose the crew to 622 mSv [268]. ISS is equipped with dosimeters and our astronauts quickly reach their life time exposure threshold. The effects of long term exposure to non physiologic levels of ionising radiations need to

be further studied together with the human body adaptation mechanisms, involving DNA repair, cell regulation and tissue responses.

Twin study The recent NASA twin study on astronauts Scott Kelly who spend one year on ISS and his twin brother, Mark Kelly, who stayed on Earth during the time of the mission, generated preliminary insights on how space travel affects the human genome. The study showed that genetic expression changed in space environment [220]. Space environment stressors are though to induce changes in DNA and RNA methylation which in turn regulate genes expression. In the twin study, most of the genetic adaptation came back to normal once back on Earth but some modifications associated with the immune system, bone formation and DNA repair persisted. Interestingly, the telomers size increased in space, advocating for a protective mechanism against space environment. Large scale omics analysis would be needed to confirm these findings.

Tissue damage Ionising radiations exposure is thought to cause cellular damage and solar flares cause DNA single/double strands breaks. Ionising radiations generate free radicals and reactive oxygen species (ROS), which in turn damage the cell structure. In a laboratory setup, beamers can simulate cosmic radiations. Studies on cell cultures have shown chromosomal damage [76] and studies on mice models showed excessive oxidative stress [166, 167]. Unfortunatly, our understanding is still poor when it comes to radiations' impact on human tissue and on the adaptive response mechanisms to repeated radiation doses [165].

Cancer risk The latest ESA scenario for a human mission to MARS counted more than 900 days for round trip to Mars with touchdown [168]. Probability projections predict a 15% fatal cancer risk if a mission in deep space exceed 180 days [75]. Ionising radiation on Earth is a well-known carcinogen and carcinogenesis also occur after exposure to space radiation [98, 76]

Central nervous system damage Ionising radiations effect on the central nervous system have been widely studied in rodent but remain poorly understood in humans. In rodents, irradiation induce a reduction in dendritic complexity and spine density[73, 196]. Degradation of neurons morphology is playing a role in the cognitive impairment reported after irradiation [74, 166]. In humans, the ISS experiment called ALTEA measured the ionising radiation levels while simultaneously recording ISS astronauts' electroencephalographic (EEG) data [267]. However, to the best of our knowledge, the results of this study have not been published yet.

Other risks Increased cataract risks has been reported in astronauts exposed to ionising radiations[65]. The increase of core body temperature by 1°C showed in astronauts on the ISS and after their return on Earth is correlated with an increase of inflammatory response. Ionising radiations could be at the origin of the inflammation [225]. A leukemia risk assessment revealed an increased risk after continuous low dose irradiation [77]. Ionising radiations may also play an important role in bone loss and inhibit osteoblast proliferation [14].

1.1.5 Isolation, confinement and extreme environment

ISS is the best model for future deep space missions, nonetheless, space analogues on Earth are often used to study the psychological effects of long-duration human space exploration missions. Some analogue stations are isolated, confined, and in an extreme environment (ICE). In this respect, Antarctic and MARS500 missions have been used to show the acute and chronic psychological strain effects of ICEs [194]. In ICEs, mood decrease during the mission and comes back to baseline toward the end of the mission [256]. This pattern is called the third-quarter phenomenon. In ICEs, the degree of confinement impacts not only psychological strain but also the amount of physical activity (i.e. sedentary). Daily physical activity has been associated with good Mood in ICEs [1]. Moreover, in ICEs, the dietary differences affect the microbiota [54].

1.1.6 Sleep as a factor of mission success

In the space industry, missions failure are often associated with human errors [152]. Long-term space flight missions raise concerns about chronic effects of space environment on the human central nervous system [68]. In deep space, physiological and psychological stress could lead to human failures. Good sleep quality and quantity might be an important factor to be looked at, since it will be key for maintaining astronaut's cognitive functions during the mission. To mitigate the risks of mission failure, we need to understand the deficit of sleep reported by ISS astronauts and we need to implement efficient countermeasures [15].

1.2 Dynamics of sleep pressure

1.2.1 Vigilance states

High sleep debt, generated by prolonged wakefulness increase sleep propensity. Inversely, wakefulness occurs once sleep debt is dissipated. Gold standard measurement of vigilance states is performed by non invasive EEG scalp recordings. EEG measures the difference of electric potential at the scalp level, which mir-

rors underlying neuronal activity and cortical oscillations [43]. According to the Fourier transform, the power spectrum of time series, represent the distribution of amplitudes for each frequency component in the signal. Vigilance states can be defined from the power spectrum of EEG recordings. Wakefulness is defined by faster frequencies and low amplitudes brain activity [23]. According to the American Association of Sleep Medicine, sleep is subdivided into non rapid eye movements sleep (NREM) and rapid eye movements sleep (REM) [25]. The deepest stage of NREM sleep, NREM3, is also called slow wave sleep (SWS) since its most prominent oscillatory component is delta (0.5-4Hz). Slow oscillations comes from the recruitment and the synchronisation of large neuronal networks [43]. Slow waves during NREM sleep is the hallmark of sleep pressure [30]. The amount of slow waves is reported as slow waves activity (SWA), which is the power of the oscillations in the delta range. During sleep, NREM and REM periods alternates and are defined as sleep cycles, each lasting approximately one hour and a half. During the sleep period, slow wave activity (SWA) decrease with the duration of previous SWS and NREM bouts are shortened. On the other hand, with the dissipation of sleep pressure, REM sleep bouts last longer toward the end of the sleep period. REM sleep is characterised by wake-like activity but accompanied by muscle atonia.

Sleep-wake regulation is controlled by two interacting processes, the circadian process and the homeostatic process [29, 30]. Sleep homeostasis regulates the sleep intensity and the circadian clock regulates its timing. Besides sleep intensity and sleep timing, the interaction between the two process controls sleep duration. However, the exact interaction mechanisms between the two processes is not fully understood yet [83]. To decrease sleep debt, both sleep duration (i.e. quantity) and sleep intensity (i.e. quality) are needed [2]. Brief awakenings and sleep fragmentation impact also sleep quality but such factors of sleep consolidation were ignored in this thesis. In this work, we choose to refer to the commonly used, "sleep intensity", as sleep quality. Therefore, sleep quality is defined by the efficient dissipation of slow wave activity (SWA) during sleep.

1.2.2 Circadian component

The circadian process is oscillating on a 24 hours cycle [78]. An aligned phase between the circadian pacemaker and the sleep-wake cycle, consolidate sleep [92]. In the absence of time cues or in the case of misleading time cues, the circadian master clock is desynchronised with the sleep wake cycle. Shift work and jet lag are common situations where the circadian clock is not aligned with the sleep-wake cycle [163]. Besides sleep timing, the circadian clock alignment also influence the duration of sleep. At the end of the sleep period, the circadian component compensate the sleep pressure decrease by maintaining sleep [30].

To study the phase alignment of the circadian rhythm, an alternative to vigilance states defined by EEG signals can be the measure of hormones' concentration in saliva (e.g. cortisol and melatonin) or the measure of neuromodulators using invasive microdialysis (e.g. adenosine). These markers oscillate in accordance with the circadian clock but do not allow a high time resolution and their impact on sleep wake behaviour is not entirely understood [90, 17].

1.2.3 Slow wave activity homeostasis

Sleep homeostasis is the regulatory process that keeps sleep pressure at an equilibrium over time [30]. The sleep homeostatic process can compensate for the reduction of sleep pressure (e.g. preceding extended sleep period or napping), by decreasing sleep propensity, sleep intensity and the total duration of sleep [**?**]. Inversely, in case of a deficit of sleep (e.g. sleep restriction or sleep deprivation), the sleep homeostasic process will increase sleep propensity, sleep quality and sleep duration. Also, brief awakenings and sleep fragmentation decrease when sleep pressure is high [111]. Prolonged wakefulness increase sleep debt and sleep debt is accumulated over the days if chronic sleep restriction is applied. Accordingly, topographical changes in SWA have been observed [95, 247, 171].

1.2.4 Theta activity during wake

Sleep pressure hypothesis The propensity to fall asleep is regulated by sleep pressure [2]. During wakefulness, microsleep episodes are observed in subjects with excessive daytime sleepiness. Microsleep behaviour is defined by a decrease of alertness, which is often tested in driving simulation experiments. From an electrophysiological perspective, microsleep episodes are defined as short periods of wake (3-14sec) where alpha-beta rhythm is replaced by theta in the EEG [33].

Sleep pressure is reflected in sleep EEG, by SWA, but can also be detected during wakefulness. Sleep pressure is low in the morning, promoting awakening. During the day, theta activity builds up with the time awake, but is also strongly influenced by the circadian clock [91, 4, 232]. In the evening, the circadian process counteract the increase of sleep pressure to sustain performances. This time of day is called " the wake maintenance zone" [45]. Accordingly, theta activity increase until after lunch time and then decrease until the evening [5, 106, 229]. After sleep deprivation theta power increase as an exponential saturating function and has been correlated to the following SWA in NREM sleep [106, 254]. Moreover, theta power in the waking EEG represents an objective measure of sleepiness [46] and is associated with alertness and cognitive performances [106, 144]. Therefore in this thesis we will refer to theta power (5-7 Hz) as a marker of sleep pressure.

In a resting state, with eyes closed, alpha oscillations (8-12 Hz) increase and become prominent in the occipital and parietal areas [23]. Alpha power is associated with the maintenance of neural network coherence in the absence of visual and sensorimotor inputs and is the marker of cortical inactivity [204]. When eyes open, alpha power decrease, together with the influx of sensory information [61]. In resting state with eyes open, cortical activity is dominated by beta activity (13-30Hz) and theta power increase with sleepiness [6]. In this work, we decided to analyse open eyes data, since theta power during closed eyes doesn't correlate with sleepiness [150].

1.2.5 Hypothesis of local sleep-like events during wakefulness

Intracortical theta oscillations during wakefulness and delta oscillations during sleep might correspond to similar neuronal "off periods" [253]. In rodents, after an intense period of activity, the neurons involved in the task are turning "off" for a short moment. Moreover, when local sleep-like events were more widespread, the task performances decreased [253]. Local sleep-like events have also been studied in humans using scalp EEG. Sleep deprivation experiments showed that the used dependant increase of theta power after prolonged wakefulness was correlated with local sleep-like events occurrences and that they might be responsible for the decrease in cognitive performances [144, 24]. Moreover, local sleep-like events appear more widespread in the evening compered to the morning and this characteristic correlates with a decrease of alertness [101].

1.2.6 Function of sleep pressure

SWS during NREM sleep is thought to be the time when neurons perform cellular maintenance [252]. SWS is a restorative period and is thought to impact the regulation of many hormones [237].

Evidences show that the repetition of a task, inducing local cortical activity during wakefulness, is mirrored by the amount of local SWA in the same cortical region during NREM sleep [142, 141]. Interestingly, local theta power increase is also task dependent and has a topographical similarity with SWA increase [144, 24]. Raising a use-dependent characteristic of sleep pressure.

An hypothesis postulate that SWA increase after sleep deprivation is mediated via the adenosinergic system [158]. Prolonged wakefulness promote the upregulation of adenosine receptors in the basal forebrain, which could modulate the long term effects of sleep debt [17].

Moreover, synaptic plasticity and sleep pressure might be closely linked. With the time awake, synaptic connections are strengthened through learning processes and SWA reflects the renormalisation of the synaptic strength [241].

This hypothesis is often used to explain why SWA is more intense at the beginning of the sleeping period and why it becomes lighter towards the end as the homeostatic pressure to renormalise the synaptic connections decreases with time spent asleep. Adequate sleep quantity and quality is required for optimal vigilance, cognitive and learning processes, which presumably are related to sleep's critical function in neuroplasticity [209, 241].

For example, chronic sleep restriction decrease alertness and cognitive performances [95, 247]. Chronic sleep restriction also increase the probability of risky decision-making [171]. In depressed patients, sleep quality is usually decreased. In this particular population, an acute sleep restriction would induce a short term antidepressant effect by increasing SWA the following night, which in turn improves the mood[120].

1.3 Sleep in space and space analogues

1.3.1 Measuring sleep in space and space analogues

To study sleep quantity and quality on the ISS and in space analogues there are different recording methods. Each of them has a different specificity and sensitivity. We reported the different options below.

Sleep diaries To measure sleep quantity, one can rely on subjective sleep questionnaires (e.g. sleep logs or sleep diaries), which report when participants went to bed and when they woke up [244, 183]. They can also report their subjective sleep quality. Unfortunately, sleep questionnaires cannot objectively report sleep quantity and sleep quality.

Heart rate and temperature Chonobiologists can measure temperature or heart beats variations along the day to reveal information about the circadian rhythm [266, 128, 264, 225].

Saliva and blood samples Chronobiology studies often take saliva samples every hour to asses the concentration of melatonin and cortisol [37, 199]. Those metabolites' concentration oscillates during the day.

Actigraphy Another common method rely on actigraphy recordings, which use a wrist accelerometer sensor [227, 70, 20, 183, 15, 109]. This device records activity each time a subject moves and assumes that inactivity periods represent sleeping periods. This method can help to assess sleep quantity and reveal information about the alignment of the sleep-wake cycle to the circadian clock. It can also be used over a long period since actigraphy is easy to implement and analyse. However, actigraphy cannot give any information about

sleep quality and is not precise enough to accurately report sleep efficiency (i.e. brief awakenings).

EEG To study sleep quality, only one method remain, EEG [198, 41, 199, 118, 128, 183, 93]. Unfortunately, this method is strenuous to setup and to analyse. Only a handful of sleep EEG has been recorded in space and in space analogues.

1.3.2 Sleep disturbances in space

The circadian clock is adjusted by the environmental Zeitgebers, day light being one of the main stimulus to the central pacemaker. On the ISS, the sun rise every 90 minutes. Moreover, astronauts are subject to very irregular schedules and workloads (e.g. launchings or dockings) [69]. As a first countermeasure, an adapted 24 hours sleep-wake routine is broadly promoted during spaceflights. However, actigraphy studies have reported a constant circadian misalignment and a mean sleep duration of 6.09 hours, when 7 to 8 hours of sleep is recommended [15, 109]. Electrocardiographic recordings were able to show a slow but reliable reentrainement of the circadian rhythm, this only at the term of the 6 months mission on the ISS. Nevertheless, a high variability remained between astronauts [264]. Recently, measurements using forehead and sternum heat flux recordings (also called Double Sensor), have been validated as a substitution method for core body temperature as a marker for circadian oscillations [225]. However, results implying a potential circadian rhythm misalignment have not been published yet.

Circadian misalignment and short sleep duration doesn't allow astronauts to fully recover and is similar to chronic sleep restriction. Astronauts sleep quality and quantity are crucial for successful space missions as sleep supports human physical and mental performances [95, 247]. Usually, sleep restriction, reduce sleep quantity, but increase sleep pressure, which should consolidate sleep and improve sleep quality. However, astronauts report subjectively bad sleep quality and use sleep promoting drugs in 11% of the nights [19, 15]. The only method to carefully study sleep quality in space would be the analysis of EEG recordings. Unfortunately, only a few studies from the space shuttles and MIR station have recorded EEG recordings, over short time periods and without a controlled environment. Sleep disturbances were systematically reported and from these studies we learned that sleep structure is different in space [128, 183, 93]. Gundel and colleagues reported a 2 hours circadian misalignment, shorter sleep duration, a less consolidated sleep and a rebound of NREM sleep in the second sleep cycle due to a shortening of the first NREM bout[128]. Dijk and colleagues reported a REM sleep rebound after spaceflight, advocating for a lack of REM sleep opportunity in space due to the shortening of sleep duration [93]. However, to draw an overall conclusion on

sleep structure during spaceflights, and assume any sleep homeostatic implications, we would need a strictly controlled circadian rhythm and more EEG recordings. Also, to understand which one of ISS environmental factors induces sleep disturbances, we would need to disentangle them and study them separately. Space analogue missions could be envisioned to mimic hypoxia, noise, temperature, microgravity, ionising radiations, confinement, isolation, stress and circadian misalignment conditions.

1.3.3 Sleep disturbances in space analogues

In space analogues, the prospect of long-term space exploration missions can be reproduced. The 520 days mission simulation to Mars, Basner and colleagues have shown that due to long term confinement and delayed communications, participants became more sedentary and had a tendency toward an increase of sleep quantity [20]. However, two participants reported chronically bad sleep quality and one participant couldn't be entrained by the 24 hours dark-like cycle and was free running. Also during the MARS 500 space mission simulation, Gemignani and colleagues reported a correlation between sleep architecture alterations and chronic stress triggered by social and environmental confinement (i.e. cortisol levels) [118]. They showed that a decrease of SWA is correlated to an increase of cortisol level (i.e. a chronic stress marker).

The first polar explorers reported "polar insomnia", which they called "the big eye". They also often complained about the isolation's psychological strain of an expedition. However, polar insomnia was only reported in the early days of Antarctica exploration, when explorers could not protect from the harsh environment of the "white continent" and had no communication with their homeland. Before the mid 19th century, there was only a few permanent station in Antarctica, nowadays spending the winter (i.e. wintering) in one of the 50 permanent Antarctic research station is more comfortable. Only a few studies from Antarctica have been recording sleep EEG. These studies reported that sleep quality decreased during winter in Antarctica and the circadian clock was misaligned with the sleep-wake cycles. Suggesting that, isolation, high altitude and constant darkness might influence sleep.

In caves with constant darkness, crewmembers can experience trouble to synchronise their circadian rhythm [182]. Interestingly artificial lights can be used as a countermeasure to entrain the circadian clock. However, unrestrained usage of artificial lights can shift the sleep-wake cycle, which will in turn not be aligned with the endogenous circadian rhythm. In Concordia during the constant darkness period, such a misalignment could occur. Moreover, Concordia station is a high altitude station and its crewmembers are exposed to chronic hypoxia. When confronted to hypoxia at high altitude, sleep quality decrease and the number of awakenings increase, although a fast acclimatisation can be observed [190, 224, 160]. A question remain when thinking of deep space explo-

ration, will humans acclimate to the new environment? If full acclimatisation cannot be envisioned, the space community has to prepare countermeasures for further space missions.

1.4 Space countermeasures

1.4.1 Physical activity

On the ISS, astronauts need to exercise for two hours every day to compensate for the lack of gravity. A bicycle ergometer, a treadmill and the Advanced Resistive Exercise Device (ARED) are used by astronauts as a countermeasure for muscle atrophy and bone density loss [263]. Besides maintaining astronauts fitness, physical exercises should be promoted for physiological reasons. In Antarctica, daily physical activity has shown to improve mood [1].

Even though long term benefits of daily physical activity are well established [210], short term effects, just after the exercise, are still poorly understood. Since sleep pressure's build up is use dependent [144], we could think of locally modulating sleep pressure with specific activities.

Fisher and colleagues showed that stereotypical wheel running in rats induced a decrease of activity in motor and premotor neurons, which in turn increased wake duration without increasing sleep pressure [108]. Following this study, it would be interesting to study if physical exercises could be envisioned as a countermeasure to decrease sleep pressure in humans.

1.4.2 Meditation

Writings and practice heritage from yoga and meditation goes back thousands of years and give us insights on how daily practice may impact brain activity. Scientific evidences and rigorous quantification of these effects remains unfortunately limited [235]. The difficulty comes from the scarcity of longitudinal studies, since an experienced practitioner is defined by daily practice of meditation for at least 60 min per day for 8 years. Moreover, it is difficult to defining a control group with similar lifestyle.

All meditation methods try to develop consciousness while improving attention and emotional self-regulation. The long term effects of meditation are thought to be gradual and they are only visible after years of daily practice [138]. Nevertheless, structural and functional cortical changes have been observed [140, 110]. However, there are many different meditation traditions with a wide range of methods and technics which can explain some results discrepancy in the literature [235].

An interesting perspective of long-term meditation, is that beside neuroplasticity, meditation could also reduce the respiration rate [260]. Moreover, the

mindfulness-based cognitive therapy (MBCT) has shown beneficial effects on syndromes varying from depression to anxiety, chronic pain and post traumatic stress disorder [221].

There is insights that long term meditation might impact sleep quality and quantity. A study showed an increase of gamma activity in the parietal areas during NREM sleep and a decrease of sleep quantity, which might indicate a decrease of sleep need in expert practitioners [103]. In sleep deprived novice meditators, alertness was improved following meditation [153]. Following this line of research and together with physical activity, it would be interesting to explore the short term meditation effects on local sleep pressure.

1.4.3 Hibernation

Inspired by the study of natural hibernators such as ground squirrels, Djungarian hamsters, bears and lemurs [134], researchers are translating this knowledge into the development of pharmacological means to control the metabolism in non-hibernators [32].

Similar to sleep cycles, hibernation is composed of repetitive cycles of torpor, arousal and sleep bouts, alternating one after another. During torpor bouts, metabolism is actively reduced, which in turn reduces the core body temperature. During the arousal bouts, core body temperature quickly return to its previous setpoint. An arousal bout is always followed by a sleep bout [79].

Sleep and torpor have a strong relation. Torpor is entered through sleep and after the arousal bout, torpor is is terminated by a period of sleep. After daily torpor in Djungarian hamsters, sleep pressure is high and proportional to the time into torpor [85]. Similar as after prolonged wakefulness, sleep pressure builds up with the time in torpor and NREM sleep after torpor has a high SWA. However, torpor EEG is silent and characterised by a low power in all frequencies which might slow down the build of sleep pressure [79, 222].

Hibernation, is a natural solution to cope with severe resource restrictions and hostile environments and hibernation should be considered for further prospects of human space exploration. Lowering the metabolic rate of astronauts would not only lead to reduced consumption of air, water and food supplies, but might also lead to lower radiation damages, improve maintenance of bone and muscle density, together with eliminating the psychological stress of a long term space travel [58, 203, 63, 127].

The induction methods for an hibernation-like state (i.e. synthetic torpor) in non hibernators can be achieved by different means. Injection of GABA agonist in the raphe pallidus has shown to induce an hibernation-like state in non-hibernators (i.e. rats) [57]. In the central nervous system, adenosine A1 receptor agonist is also inhibiting thermogenesis [243, 148]. Furthermore, Hydrogen sulfide (H2S) injections, reduce the ATP synthesis by acting on

mitochondrion and seems to have anti-apoptotic and antioxidant proprieties which reduce organ damage during hypothermia [97]

1.5 Aims of this thesis

In this thesis we subdivided our work into three lines of research with three associated research papers, presented here as chapters. First we studied sleep pressure markers in five astronauts on the ISS and we investigated sleep pressure marker's association with cognitive performances. Second, we investigated sleep pressure markers in twelve participants during a eight months space analogue mission on Earth, at the Concordia Antarctica research station. We studied the association of sleep pressure markers with psychological strain and investigated the benefit of physical activity as a countermeasure. Third, we reviewed the mechanisms of hibernation and confirmed the attractiveness of an hibernation-like state for human spaceflight beyond Earth orbit.

Chapter 2

Local sleep-like events during wakefulness and their relationship to decrease in alertness on the International Space Station

Local sleep-like events during wakefulness and their relationship to decrease in alertness in five astronauts on the International Space Station

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Own contribution: *designed the data analysis, negotiated the data sharing agreement, managed the ESA Ariadna grant, analysed the data, wrote and edited the manuscript*

Publishing status: *Accepted with NPJ Microgravity*

Abstract

Adequate sleep quantity and quality is required to maintain vigilance, cognitive and learning processes. A decrease of sleep quantity preflight and on the International Space Station (ISS) has been reported. Recent countermeasures have been implemented to better regulate sleep opportunities on ISS. In our study, astronauts were allocated enough time for sleep the night before the recordings. However, for proper sleep recovery, the quality of sleep is also critical. Unfortunately, data on sleep quality have yet to be acquired from the ISS. Here, we investigate sleep pressure markers during wakefulness in five astronauts throughout their six month space mission by the mean of electroencephalographic recordings. We show a global increase of theta oscillations (5-7Hz) on the ISS compared to on Earth before the mission. We also show that local sleep-like events, another marker of sleep pressure, are more global in space (p¡0.001). By analysing the performances of the astronauts during a docking simulation, we found that local sleep-like events are more global when reaction times are slower (R^2=0.03, p=0.006) and there is an increase of reaction times above 244ms after two months in space (p=0.012). Our analyses provide first evidence for increased sleep pressure in space and raise awareness on possible impacts on visuomotor performances in space.

Keywords

Sleep, local sleep, Space, astronauts, microgravity, neuroscience, electroencephalographic data, alertness, reaction time, cognitive task, docking simulation.

Introduction

Sleep is regulated by two oscillatory processes: the circadian process and the homeostatic process. The circadian process oscillates on a near 24 hour rhythm and defines the sleep inclination periods [92]. Under normal conditions, the circadian process is synchronised with the light/dark cycle. Astronauts witness short sunrises 16 times a day, since the International Space Station (ISS) orbits Earth every 90 minutes. As a countermeasure, an artificial 24 hour sleep/wake routine is established to align astronauts' circadian rhythms with Coordinated Universal Time (UTC). Despite this countermeasure, it has been reported that a majority of the ISS crew members are using sleep promoting medication in response to a sleep deficiency, before and during their mission [15].

Even though astronauts were allocated enough time to sleep in space, sleep quality might not be sufficient to fully dissipate sleep debt. In space, sleep quality might be impacted by external factors such as microgravity, confinement, circadian misalignment, chronic stress, temperature, light and noise disturbances [239, 68]. So far, experiments have shown sensorimotor and neurovestibular adaptation to the space environment [265, 62, 130]. Yet, no conclusion on changes in the underlying mechanisms regulating sleep in space could be made. Most related ISS studies focus on sleep quantity and chronobiology [264, 109], because during human spaceflight all-night sleep recording is hard to achieve. Only a few reports from Space Shuttles and the early days of human space stations feature sleep electroencephalographic (EEG) data [128, 183, 93].

Regulated separately but operating in parallel with the circadian process, the homeostatic process, represented by sleep pressure (i.e. sleep need), builds up with the time spent awake. Scalp EEG measurement allows real-time recordings of cortical activity and is currently the gold standard for sleep quality analysis. EEG markers of high sleep pressure can be observed during wakefulness, by increased theta activity (5-7 Hz) and while asleep by an increase of slow wave activity (SWA) (0.5-4 Hz) [47, 106, 30]. Although, theta activity increases with the time awake, a strong circadian modulation can be observed [4, 5, 229]. Dynamic changes in SWA's topographical distribution and a frontal increase in the number of high amplitude slow waves, have been observed after chronic sleep restriction [205]. Moreover, the local regulation of SWA seems to be closely related to the capacity for neuroplastic changes [241]. Recently, it has been shown that similar local sleep-like events can be observed in awake rodents [253]. Using high density scalp EEG, local sleep-like events have also been studied in awake humans alongside the increase of sleep pressure [102] and after a period of sleep deprivation [144, 24].

Here, we analyse how astronauts' local sleep-like events during wakefulness are impacted throughout their six month space mission and we investigate if

they are related to visuomotor performances. This analysis is based on high-density wake EEG data collected between 2011 and 2013 on the ISS, as part of the Neurospat experiment [55].

Results

Despite similar sleep and wake quantity, theta power changes from Earth to space recordings

All astronauts were recorded while repeatedly docking a simulated Soyuz vehicle to the ISS for 70 minutes in three conditions: on Earth before the mission (Earth: -62.8 ± 8.0 days), about two weeks after the launch (space1: 10.4 ± 1.9 days) and about two months after the launch (space2: 56.0 ± 3.4 days). Sleep quantity the night before the recording session was not different across conditions (Earth: 6.4 ± 0.4, space1: 7.0 ± 0.6, space2: 6.8 ± 0.4 hours, linear mixed-effects model with Earth/space1/space2 as a fixed effect and different random intercepts for each astronaut, $F(2,12)=0.40$, $p=0.679$, $n=15$ recording sessions)(Fig. 2.1.a) and the duration of wakefulness before the recording was not different across all three conditions: Earth (293 ± 45), space1 (327 ± 77) and space2 (367 ± 76 minutes) (linear mixed-effects model with Earth/space1/space2 as a fixed effect and different random intercepts for each astronaut, $F(2,12)=0.60$, $p=0.564$, $n=15$ recording sessions) (Fig. 2.1.b). Similarly, no significant difference was shown for the time of the recording and the wake up time (Supplementary Fig. 2.8). In the first step we assessed theta power's topographical distribution across the scalp. Theta power showed a similar distribution across the three conditions (Fig. 2.2.a). In the majority of astronauts we observed a global increase (i.e. when at least 50% of the electrodes are involved) in theta power from Earth to space (in five astronauts out of five for space1 and four out of five for space2). However, by comparing Earth to space no cluster of more than two electrodes showed a significant increase of theta power (df=4, white dots uncorrected p-values ¡ 0.05, n=5 astronauts) (Fig. 2.2.b) and a high variability can be observed across astronauts (Fig. 2.2.c).

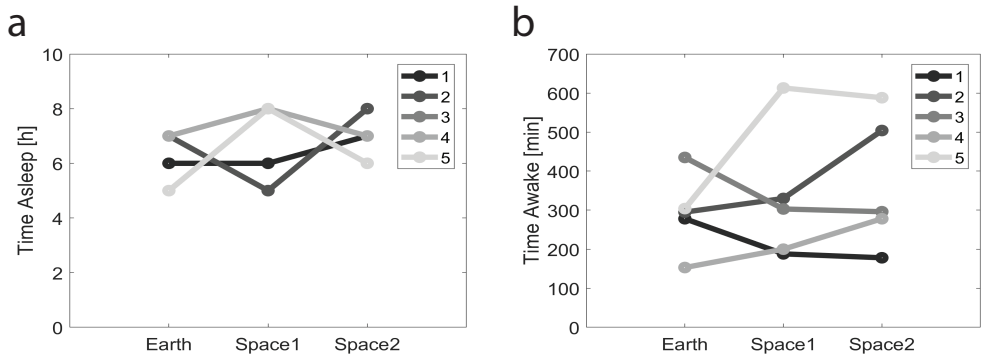

Figure 2.1: Three conditions: one on Earth, two in Space. (a) For each astronaut, the quantity of sleep the night before the recording is not different across conditions. (b) For each astronaut, the time they have been awake before the beginning of the recording is not different across conditions. (n=5 astronauts)

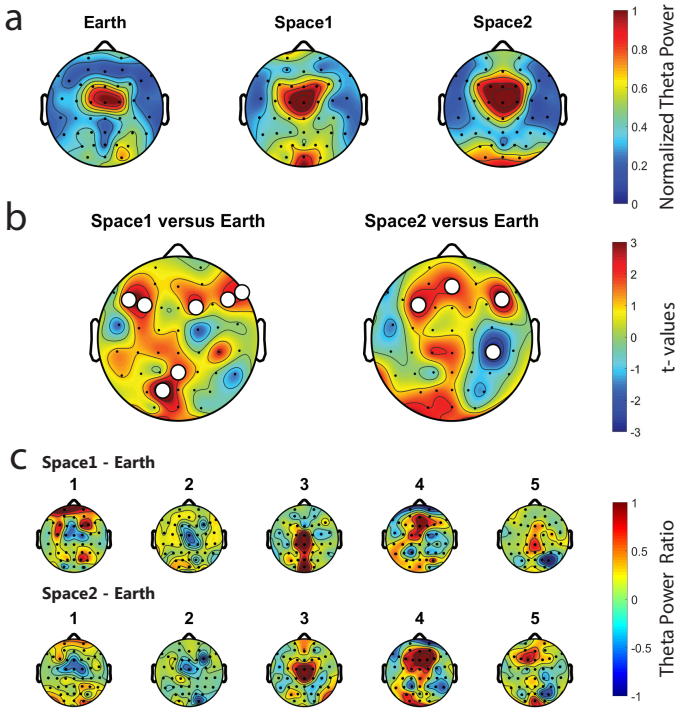

Figure 2.2: Theta power topographical distribution across the recording conditions. (a) Normalised theta power for Earth, space1 and space2. Consistent central distribution of the theta power in all conditions. (b) In space1 and space2 compared to Earth, theta is globally increased for a majority of astronauts. Red colour indicates an increase of theta power in space compared to Earth (paired t-test, t-values, white dots uncorrected p-values ¡ 0.05). (c) Space1-Earth and space2-Earth differences in theta power for each astronaut (1-5). Differences in theta power from space to Earth are observed but no clear topographical pattern emerges. (n=5 astronauts)

Local sleep-like events' amplitude and globality changes from Earth to space recordings

To investigate sleep pressure markers during wakefulness, we detected local sleep-like events. i) We first defined three areas of interest along the anterior-posterior axis (i.e. frontal, central and parietal)(Fig. 2.3.a). ii) Then, we defined specific detection thresholds according to theta oscillations within each of these areas (Fig. 2.3.b). Specific thresholds were computed for each condition (i.e. Earth/space1/space2) and averaged within each astronaut. We show that the number of local sleep-like events did not vary by topographical areas (linear mixed-effects model with frontal/central/parietal as a fixed effect and different random intercepts for each astronaut, $F(2,42)=1.844$, $p=0.171$, n=45 measures), nor by recording conditions (linear mixed-effects model with Earth/space1/space2 as a fixed effect and different random intercepts for each astronaut, $F(2,42)=0.28$, $p=0.758$, n=45 measures) (Fig. 2.3.c). iii) To look further into the characteristics of local sleep-like events, we calculated the amplitude between the negative peak and the positive peak for each local sleep-like event (Fig. 2.3.d). We also examined how many electrodes were involved in each event, defining this latest property as the globality of an event (Fig. 2.3.e). Our results show that the amplitude varies across topographical areas (linear mixed-effects model with frontal/central/parietal as a fixed effect and different random intercepts for each astronaut, $F(2,42)=5.73$, $p=0.006$, n=45 measures). The amplitude of the local sleep-like events is higher in the frontal area compared to the central area ($z-score : +1.41 \pm 0.42$, $t=3.43$, $df=42$, $p=0.002$). We also calculated the differences in globality of the detected events and we found that globality varies across conditions (linear mixed-effects model with Earth/space1/space2 as a fixed effect and different random intercepts for each astronaut, $F(2,42)=21.11$, $p<0.001$, n=45 measures). Specifically, in space local sleep-like events are more global compared to Earth (Earth to space1: $+4.06\% \pm 0.66$, $t=6.13$, $df=42$, $p<0.001$, Earth to space2: $+3.26\% \pm 0.66$, $t=4.92$, $df=42$, $p<0.001$).

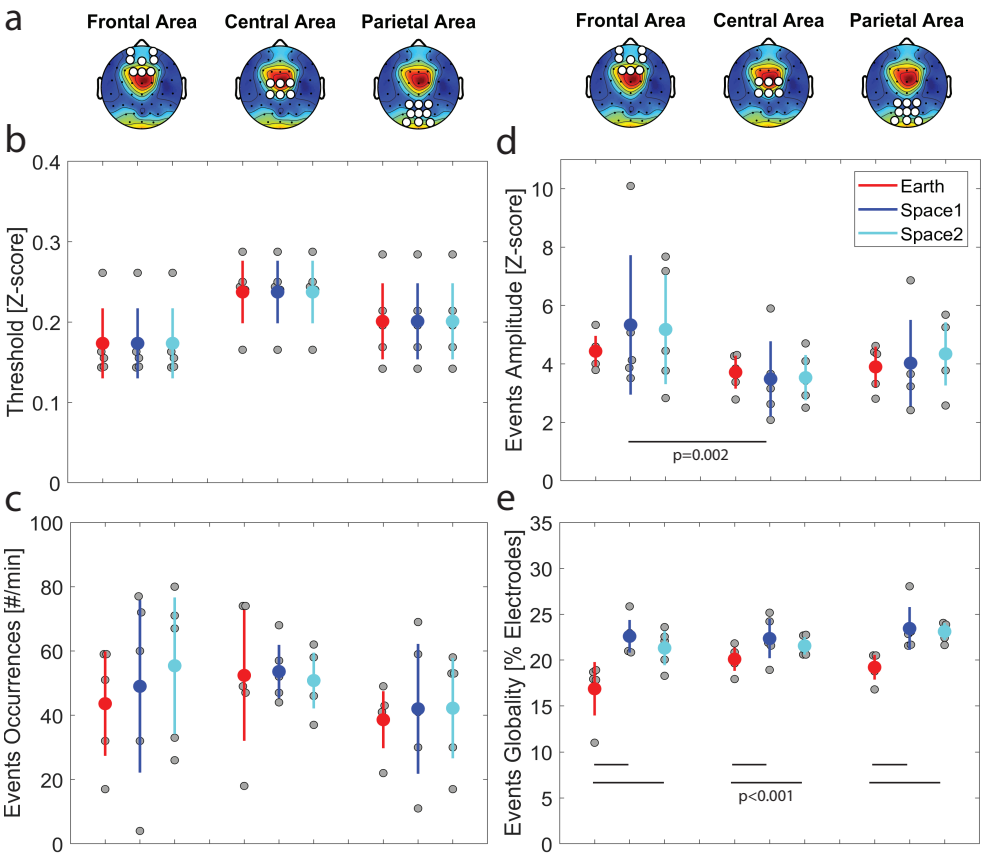

Figure 2.3: Local sleep-like events' properties. (a) Topographical distribution of theta power in space2. The white dots define the three non overlapping areas (frontal, central and parietal) used for the detection of local sleep-like events. (b) The voltage at each channel is standardised by z-score transform. A detection threshold is defined for each astronaut within each area (i.e. frontal, central or parietal area). Each grey dot represents the mean value for one astronaut and the $mean \pm sem$ across astronauts are colour marked as a dot and a line respectively. (c) No difference is the number of local sleep-like events selected in each area and across conditions. (d) Increase of the amplitude for local sleep-like events in the frontal area compared to the central area. (e) Increase of the globality for local sleep-like events from Earth to space. (n=5 astronauts)

Alertness and cognitive performances changes from Earth to space recordings

The visuomotor task performed by the astronauts on the day of the experiment, which involves piloting and preparing the docking of a simulated Soyuz vehicle to the ISS, had two outcomes: 1) the astronauts succeeded in recovering and docking the simulated Soyuz vehicle or 2) they failed (Fig. 2.6). By looking into the local sleep-like events' properties, the outcomes of the task could not be predicted based on the amplitude (generalised linear mixed-effects model, assuming a binomial response distribution, with events' amplitude as a fixed effect and different random intercepts for each astronaut, F(1,453)=0.01, p=0.951, n=455 trials), nor by the globality of the events (generalised linear mixed-effects model, assuming a binomial response distribution, with events' globality as a fixed effect and different random intercepts for each astronaut, F(1,453)=0.02, p=0.893, n=455 trials)(Fig. 2.4.a,b). When carefully looking at reaction times, we observed a variation in the amount of reaction times above 244 ms (i.e. the median reaction time) from Earth to space (linear mixed-effects model with Earth/space1/space2 as a fixed effect, different random intercepts for each astronaut and by-astronaut random slopes for the fixed effect, F(2,12)=4.52, p=0.034, $n_{Earth} = 5$, $n_{space1} = 3$, $n_{space2} = 4$ astronauts)(Fig. 2.4.c). Specifically, the percentage of reaction times above median (reaction time) increased by 18.03% $\pm$ 6.05 in space2 compared to Earth (t=2.98, df=12, p=0.012). Moreover, looking at all reaction times across recording sessions, excluding outliers, it appears that local sleep-like events are more global during slower reaction times (linear regression model with events' globality as a fixed effect: R^2=0.03, F=7.7, df=235, p=0.006, n=237 trials)(Fig. 2.4.d).

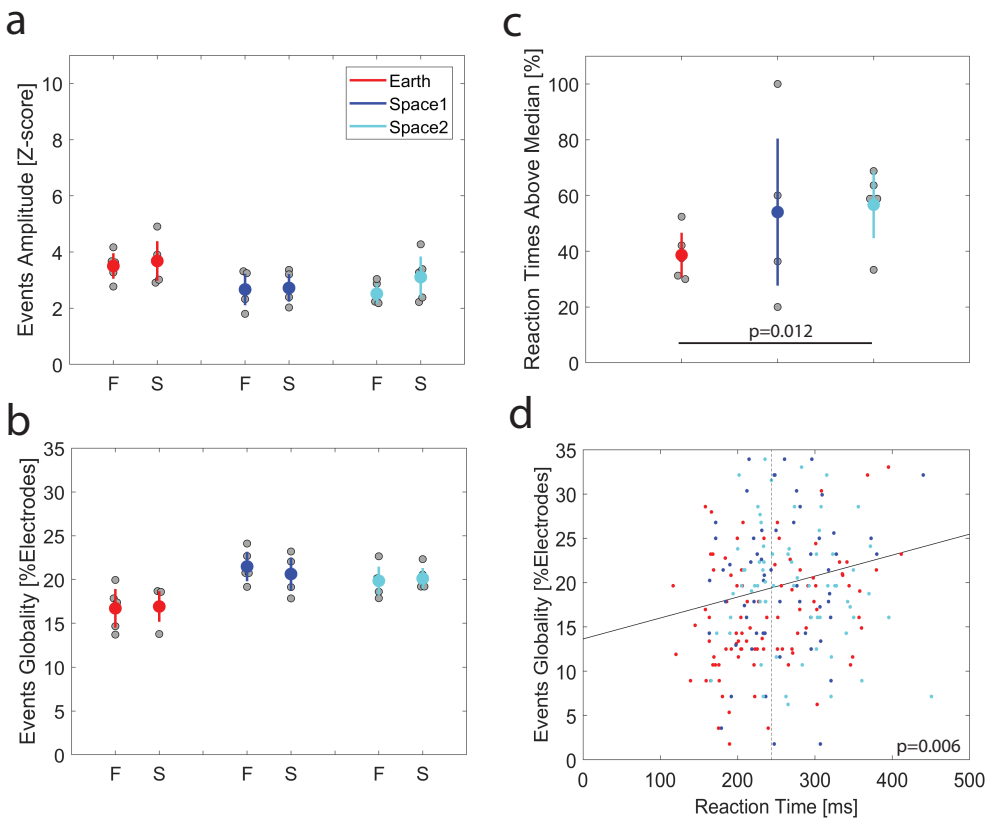

Figure 2.4: Local sleep-like events impact on cognitive tasks and alertness. (a)(b) During the 10 seconds of the recovery manoeuvre, the astronauts either Failed (F) or Succeeded (S) to dock the simulated Soyuz vehicle. The two outcomes of the visuomotor task could not predict the local sleep-like events' properties. Each grey dot represents the mean value for one astronaut and the $mean \pm sem$ across astronauts are colour marked as a dot and a line respectively. (c) Reaction time until first movement while performing the recovery manoeuvre. There is a higher percentage of above median reaction times in space2 compared to Earth. (d) The full line illustrates the positive correlation between the local sleep-like events' globality around the starting point of the recovery manoeuvre (-250 ms 500 ms) and the reaction times across all trials. The median reaction time is marked by a dotted line at 244 ms. (n=5 astronauts)

Discussion

In this study, we observed a global increase of theta power in space compared to the Earth condition without any consistent topographical pattern. To further analyse sleep pressure markers and reveal potential negative effects of the ISS environment on sleep, we detected local sleep-like events. We showed that local sleep-like events are more widespread over the scalp (i.e. increased globality) in space compared to Earth. While analysing the outcomes of the visuomotor task, we observed an increase of above median reaction times after two months in space compared to Earth. By correlating sleep pressure markers with astronauts' performances, we found a positive association between the globality of the local sleep-like events during the first movement and reaction times. However, local sleep-like events were not associated with the astronauts' docking performances.

As expected from an electrophysiological marker of sleep pressure, numerous studies showed increased theta power after sleep deprivation [4, 5, 106, 229]. Thus, our observation that theta power was increased across the cortex in space compared to Earth might indicate increased sleep pressure in space. Studies using sleep deprivation also found that the increase in theta power was most pronounced over frontal midline areas [106, 229]. With only five astronauts, our study might have been under-powered to detect topographical changes in theta power. The individual topographical increase of power, however, was quite reproducible between Earth and space1 and Earth and space2, which indicates good signal quality across conditions. The effects of sleep restriction compared to sleep deprivation are typically less pronounced on the electrophysiological level [170]. Since the astronauts got about 6-7 hours of sleep the night before the recordings, we would expect rather small changes in theta power, further limiting the likelihood to see local changes in theta power. However, our knowledge of the sleep-wake history is limited to the night before the recording.

Studies in rats and humans have shown that increased sleep pressure is associated with more widespread local sleep-like events [253, 144, 24, 102]. Thus, our observation that local sleep-like events are more widespread in space compared to Earth further supports increased sleep pressure in space. Increased sleep pressure is typically also associated with slower reaction times, a behavioural measure of a decrease in alertness [18]. Although we found no change in general docking performance across the three conditions, we found a significant increase in above median reaction times in space. This observation is in agreement with the increased sleep pressure measured in space.

Interestingly, the studies investigating local sleep-like events have presented an association with task performance [253, 144, 24, 102]. Specifically, temporal proximity of task execution to more widespread local sleep-like event was associated with performance deterioration [253**?**]. We found a similar,

though very weak, relationship in that globality of local sleep-like events was associated with slower reaction times. What compels a local sleep-like event to involve larger cortical areas is not fully understood. One explanation might be that more widespread local sleep-like events may represent an increased level of network synchronisation as a result of the homeostatic build-up of learning related synaptic strength [241]. Even though effects of increased sleep pressure on cognitive performances has been well established [247, 171], direct implication of local sleep-like events' globality on cognitive tasks would need to be further explored in a larger sample population.

Electrophysiological and behavioural markers indicate increased sleep pressure in space. Sleep pressure is regulated by two factors, a homeostatic process and circadian rhythms. What underlays this increased sleep pressure in space is difficult to answer with our data. The homeostatic build up of sleep pressure is directly dependent on previous sleep and wake duration [30]. Thus, we took great care in selecting recording sessions for which both the duration of sleep the night before as well as the duration of wakefulness before the recording session were not significantly different across conditions. To do so we had to exclude a large number of pre-flight recordings, which means that in follow-up experiments, the time at which the astronauts are recorded should be better controlled. In the Neurospat experiment each astronaut was allocated 8.5 hours for sleep the night before the recording and sleep quantity the night before the recording was not systematically different across the conditions. Accordingly, an increase of sleep pressure in space is likely not due to a loss of sleep quantity the night before the recording. However, experimental evidence using actigraphy data indicates that astronauts experience a lack of sleep quantity on Earth before the mission and on the ISS [15, 264, 109]. In our study we cannot exclude that astronauts were partially sleep restricted since we have no actigraphy recordings nor sleep diaries for the week before the recordings.

As introduced, sleep recovery is not only dependent on sleep quantity but also on sleep quality. Thus, our observation of increased sleep pressure could be due to a loss of sleep quality in space. To confirm a decrease of sleep quality we would need night EEG recordings. Unfortunately, such measurements were not available in the Neurospat experiment. Multiple factors could impact sleep quality in space: microgravity, confinement, chronic stress, temperature, light and noise disturbance[239, 68]. Unfortunately, the direct impact of each single space environmental factor on sleep quality could not be assessed within this study due to the limits of the experimental protocol. We have only two recording sessions in space and we are unable to show if our findings are impacted by microgravity per se (i.e. short-term effects). Thus, in microgravity, potential differences in the underlying mechanisms regulating local sleep-like events require further investigation.

Finally, circadian rhythms could also impact measures of sleep pressure [91, 4, 5, 232, 30]. Indeed, astronauts have been shown to be often misaligned with their circadian clock during their mission [264, 109]. However, to assess such circadian alignment, we would need constant actigraphy recordings or sleep diaries along the mission. Unfortunately, none of these measurements were available for all the astronauts participating in the Neurospat experiment. To limit confounding effects of misalignment, the Neurospat experiments were not performed in the 48 hours following air travel that involved a change of more than 4 time zones, nor following work shifts inducing more than 4 hours of time shift, nor on the day after imposed sleep deprivation. Moreover, we were selective in choosing the included recording sessions. In regard to the time of day at which the recording took place, we made sure that they were not significantly different across the conditions. Nevertheless, as we do not have any information about circadian alignment the days before the recordings, we cannot fully exclude circadian misalignment.

With all the limitations in mind, our analysis provide initial evidence for increased sleep pressure in space. It would be interesting in future studies, involving more astronauts, to see whether these findings are related to task performance. Sleep quality might be an important factor to consider because it will be key for maintaining astronaut's cognitive functions and improving missions' success rate.

Methods

Participants and experiment

Participants and experiment

Five male astronauts (53 ± 1.6 years old) took part in the Neurospat experiment (AO-2004, 118). Written informed consent was obtained prior to participation. The experimental protocol was approved by the European Space Agency's Medical Board (ESA-MB) and the NASA Johnson Space Center Institutional Review Board (NASA-IRB). To ensure comparable levels of sleep quantity the night before the recordings, a sleep questionnaire was filled out by astronauts. Astronauts were allocated 8.5 hours for sleep the night before the experiment and we excluded all recordings where astronauts reported sleeping less than five hours.Neurospat experiments were not performed in the 48 hours following air travel that involved a change of more than 4 time zones, nor following work shifts inducing more than 4 hours of time shift, nor the day after imposed sleep deprivation, nor after a highly strenuous physical or mental activity such as extravehicular activities, centrifuge training, vestibular counter-measures experiments. Astronauts were instructed to maintain their normal consumption of caffeine but were not allowed alcohol nor medication 16 hours before the experiment. Although Neurospat's principal investigators

asked to perform the experiments at the same time of day, preferably the morning, recordings took place at variable times along the day, between two and ten hours after awakening. In this study, we defined the recording conditions with the label Earth for the recordings on Earth and space1/space2 for the recordings on the ISS. During each Neurospat recording session, the astronauts had a resting state period, a visual orientation task and a visuomotor task to perform. Prior to Earth recordings, the astronauts had two training sessions on Earth to get familiar with the tasks. These two training sessions were not used in this analysis. Then, they had two recordings on Earth before the mission for each astronaut. Out of the two Earth recording available in the Neurospat experiment, we included in our analysis only the Earth recording with a minimum difference in time after awakening compared to the space recordings. Finally the astronauts had two recordings on the ISS. We had to discard post-flight recordings because they were recorded on another EEG system (Advanced Neuro Technology) at the Johnson Space Center (Houston, USA).

Wake EEG recordings

Each participant, for each session, was recorded with 58 EEG electrodes with the multi-electrode electroencephalogram mapping module (MEEMM) from the European physiology module placed on the ISS Columbus module, at the European Astronaut Centre (Köln, Germany) or at Star City (Moscow, Russia). In addition to the 58 EEG electrodes (10–20 electrode system EEG cap), three electrooculogram (EOG) (allowing horizontal and vertical EOGs), one electrocardiogram (ECG) and one electromyogram (EMG) (recorded at the first interosseous muscle of the right hand) were recorded. Continuous wake EEG was recorded for 70 minutes during each Neurospat session at a sampling rate of 1116 Hz (0.01–558 Hz band width). Scalp electrodes' impedance were measured and kept below 5 KΩ. For all recordings, the reference was placed on the right ear lobe. EOG, EMG, ECG and derivation P5 and P6 were excluded from further analysis. On Earth, the astronauts performed the experiment seated at a table. On the ISS Columbus module they were free floating with a secured loose-fitting leash around the waist and attached to the European Physiology Module rack.

EEG pre-processing

EEG data pre-processing was performed in Matlab (Version R2017b) using EEGLAB toolbox scripts (Version 14) [86] and additional custom made scripts. EEG data were pass-band filtered [0.1–48 Hz] and down sampled to 512 Hz. The signal was recorded as the difference of potential between the electrodes of interest and the right earlobe (i.e. earlobe referencing), which dampened the amplitudes of all oscillations close to the reference point and ultimately induced an asymmetry toward the left hemisphere. To correct for this effect, we transformed the data by subtracting the average activity across all elec-

trodes (i.e. average referencing). A first Independent Component Analysis [87] was performed to remove ocular, muscular, and electrocardiographic artifacts (Earth: 2.6±0.7, space1: 1.0±0.0, space2: 1.8±0.5 components rejected) as defined by Hulse and colleagues [143]. Using the EEGLAB graphical user interface, all movement artifacts in the signal were marked by visual inspection and removed (Earth: 55.3 ± 2.2, space1: 60.6 ± 2.8, space2: 64.7 ± 4.4 minutes of recording remaining). The power spectrum was computed for each channel and outliers containing high muscle artefacts (20-30Hz) were excluded from the dataset (Earth: 2.0±0.7, space1: 1.6±0.8, space2: 0.8±0.3 channels rejected) [102]. A Second Independent Component Analysis was performed on cleaned data to further remove ocular, muscular, and electrocardiographic artefacts (Earth: 4.2 ± 0.9, space1: 4.0 ± 0.7, space2: 4.4 ± 0.8 components rejected). For each subject, rejected channels were interpolated. To the best of our knowledge, the prospect of an impedance difference at the electrodes between Earth and space has never been studied. With the assumption that such a difference could occur due to microgravity (e.g. electrical conduction differences), we prevented any effects of the recording montage on the signal by using a z-score transformation. Moreover, to improve the signal to noise ratio in the power spectrum analysis, we performed a phase-rectified signal averaging (PRSA) [161]. PRSA allows superimposing of the oscillations to create interference and hence reduce the weight of acute noise generators in the signal. The power spectral density was estimated using the Welch's averaged periodograms with a four second Hamming window and a frequency resolution of 0.125 Hz. In each frequency bin, the power at each channel was normalised by the average power over the scalp. The theta power band was computed between 5 and 7 Hz, to stay distant from astronauts' alpha peak (8-10 Hz)[61]. As in previous work [144], we define a global increase when more than 50% of the electrodes were involved.

Local sleep-like events detection

To look for evidence of local sleep-like events during wakefulness we combined existing detection methods [172, 144, 24, 177, 144, 102]. We used the SWA-Matlab toolbox developed by Mensen and colleagues with the following parameters [177, 175] (Fig. 2.5). The EEG channels within three non overlapping areas (frontal electrodes: Fp1, Fp2, AF3, AFz, AF4, F1, Fz, F2, central electrodes: FC1, FCz, FC2, C1, CPz, CP2 and parietal electrodes: CP1, CPz, CP2, P1, Pz, P2, PO3, POz, PO4) were averaged and the three outcomes were filtered within the theta band using a second order Butterworth band pass filter. A threshold was set at two times the median deviation from the median signal for each area reference signal. To correct for potential remaining slow drifts in the EEG signals, we chose to detect local sleep-like events using the minimum negative point between two maximum peaks oscillating within theta oscillations, instead of the commonly used minimum negative point between two consecutive zero crossings. All negative peaks on the refer-

ence signal below this relative threshold were detected and marked as a local sleep-like event (7019 ± 488 events per recording session). To study the size of each local sleep-like event over the scalp, the event's globality was computed by cross correlating the reference signal with each channel across the scalp within the theta range, looking for similar oscillations within a 50 ms time window. For each correlation above 95%, the corresponding channel was marked as involved in the event. As an additional layer of security, we unmarked isolated channels which could represent artifacts by applying a cluster test [176]. By defining a 50 ms time window to assess how many electrodes are involved in a local sleep-like event, we assumed that the theta waves are travelling over the scalp at least twice faster than slow waves [172, 102]. The number of areas of interest over the scalp defined the sensitivity of our detection algorithm. By choosing three areas of interest, we targeted only events within one of these areas and by averaging the signals within these areas, we targeted events involving at least a few electrodes. To refine the detection of events in specific cortical areas, we would need higher density EEG recordings, exceeding the current 58 electrodes. Moreover, average referencing will subtract the signal of the neighboring electrodes which might prevent our algorithm from detecting local sleep-like events involving only one electrode. Eventually, average referencing will induce a bias toward the detection of more global events compared to earlobe referencing. We further assessed the density of local sleep-like events per minute of recording and the amplitude from the negative peak to the following positive peak, measured at the channel with the median slope across all channels involved in the corresponding event.

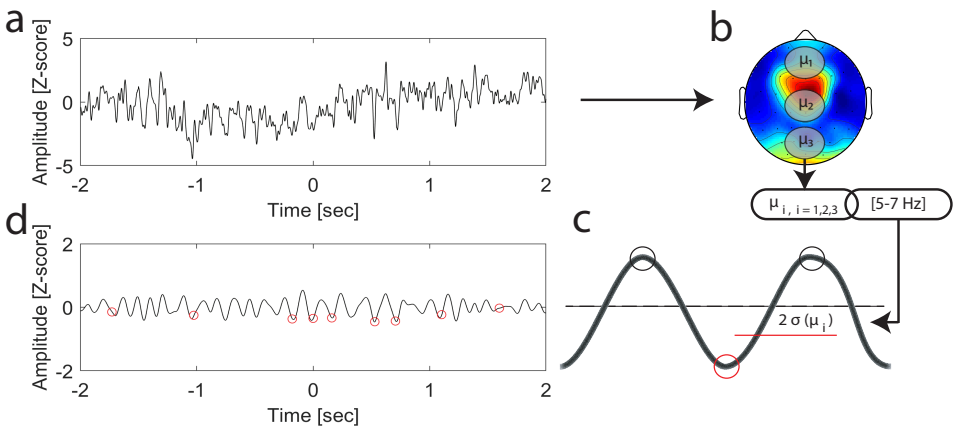

Figure 2.5: Local sleep-like event detection method. (a) Difference of potential at the derivation C3. (b) Signal average within three non overlapping areas (frontal, central and parietal) and filtered within the theta band. (c) Detection threshold at two times the median deviation from the median signal within each area. All negative peaks on the reference signal below this relative threshold were detected and marked as a local sleep-like event. (d) Red circles mark the local sleep-like events involving the derivation C3.

Visuomotor task

During the experimental protocol, the astronauts had to perform a visuomotor task that lasted 30 minutes (Fig. 2.6). The astronauts were told to look straight ahead at a laptop screen through a facemask to remove any external visual cues (Fig. 2.7). The astronauts were looking at a virtual display on a computer screen, simulating randomly two scenarios: one piloting the Soyuz vehicle, preparing a docking to the ISS (ISS being the target in this case) or the second while being within the ISS, preparing remotely the docking of the Soyuz vehicle (the Soyuz being the target in this second case). There were 80 trials per session (40 for each scenario). The 80 trials were divided in four blocks, which allowed the astronauts to take a break before starting the next block of 20 trials. At the beginning of each trial and for two seconds the astronauts saw first their own spaceship and then their target. Following this, the target deviated from its nominal straight-ahead position for another two seconds. Throughout this first period, the astronauts were asked to observe their target (ISS or Soyuz vehicle) without performing any movement. Six seconds after the beginning of the trial, the centre of the target changed from white to grey, which meant that the astronauts were required to take control of the spaceship and perform, as quickly as possible and in less than seven seconds, the recovery manoeuvre toward the target by controlling a joystick with their right index finger. Once the docking position was reached, the astronauts were asked to confirm their attempt by pressing a button with their right thumb. The centre of the spaceship changed from white to blue if they successfully (S) docked the spaceship or to yellow if they failed (F). The next trial would start two seconds later. The astronauts had to perform the same experiment repeatedly during each recording session, bringing the total number of artifact free trials to 455 (Fail: 10.80 ± 1.00, Succeed: 21.27 ± 1.35 trials per session and per astronaut). The time point from which the astronauts were allowed to recover the trajectory of the spacecraft until the first movement recorded with the joystick was defined as reaction time. Only 15 reaction times were above 500 ms. They were considered as outliers and discarded from our analysis. Reaction times below 100 ms were due to anticipated movements (i.e. false start) and also discarded, reducing the total number of trials to 237 [18]. The time interval to look for local sleep-like events was defined as 250 ms before stimulus (i.e. motor action planning) and 500 ms after stimulus presentation (i.e. maximal reaction time) [202].

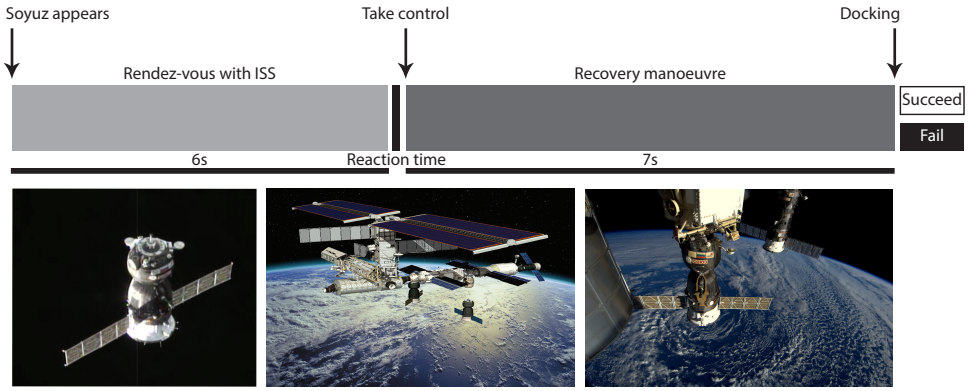

Figure 2.6: Visuomotor task experimental protocol. Rendez-vous with ISS (first six seconds) and recovery manoeuvre (following 7 seconds). The time when the astronauts are allowed to recover the simulated Soyuz vehicle until they take control of the spacecraft with their first movement is defined as reaction time. Photographs used from ESA, ESA-David Ducros and ESA/NASA with permission (photographs from left to right).

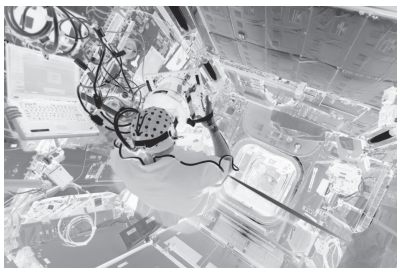

Figure 2.7: Astronaut performing the Neurospat experiment on the ISS Columbus module. Photograph used from ESA/NASA with permission.

Statistics

Data points were reported as $mean \pm sem$. For topographical analysis we plotted t-values for the two-tailed paired Student's t-tests (uncorrected p-values) and we used a non-parametric permutation test (coefficient of variation=2.757, 2^5 permutations, n=5 astronauts) for cluster correction [142, 101], defining the minimum cluster size of significant neighbouring electrodes for a pattern to be significant. In our analysis at least 3 neighbouring electrodes needed to be significant to be reported as a significant effect. For repeated measures time series, we used mixed-effects analysis to model repeated measures. First we assessed normal distribution with a quantile-quantile plot. For normal distributions, we performed a linear mixed effects analysis of the relationship between the response variable and fixed/random effects. For non-normal distributions (e.g. binomial distributions), we used a generalised linear mixed effects analysis. We used the restricted maximum likelihood estimate method to fit the model and choose the best model based on Bayesian information criterion results. Visual inspection by quantile-quantile plot of the residuals confirmed that homoscedasticity and normality were respected. The influence of the fixed effects on the model were determined by F-tests (F(degrees of freedom in the numerator, degrees of freedom in the denominator)=F-value, p=p-value). We finally reported the estimated differences ($mean \pm sem$) between repeated measures for each fixed effect, together with the two-tailed paired Student's t-tests results (t=t-values, df=degrees of freedom, p=p-values). When no fixed effects could help to fit the model (i.e. best model is the intercept only model), we reported the non-significant results for the F-test assessing the influence of each fixed effect candidate on the model. If the variable of interest is best modelled by a unique fixed effect without random effects, we used a linear regression model to obtain a R^2 adjusted value which indicates how much of the total variation can be explained by the fixed effect. Then, we completed a F-test (F-value, df=degrees of freedom, p=p-value) with the null hypothesis that the slope of the model is equal to zero. All statistical analysis were performed in Matlab.

Data availability

All relevant data will be available from the corresponding authors upon request and after approval from the European space Agency Medical Board (ESA-MB) and the NASA Johnson space Center Institutional Review Board (NASA-IRB).

Code availability

All relevant scripts will be available from the corresponding authors upon request.

Acknowledgements

This work was supported by the European Space Agency and the Luxembourg Government within the framework of the Ariadna study 17-9701. It was also supported by a research grant from the University Research Priority Program of the University of Zurich (Clinical Research Priority Program Sleep and Health). We thank Maurice Marnat at the French institute for space medicine and physiology (MEDES) for his help in unravelling missing information.

Author contributions

G. Cheron, A.M. Cebolla and M. Petieau conceived the experiment and performed the measurements. G. Petit, R. Huber and L. Summerer designed the data analysis study. G. Petit performed the data analysis and wrote the manuscript. S. Fattinger and R. Huber gave data analysis support. All authors contributed to the scientific discussion and manuscript revisions.

Competing Interest

The authors declare that they have no competing interests.

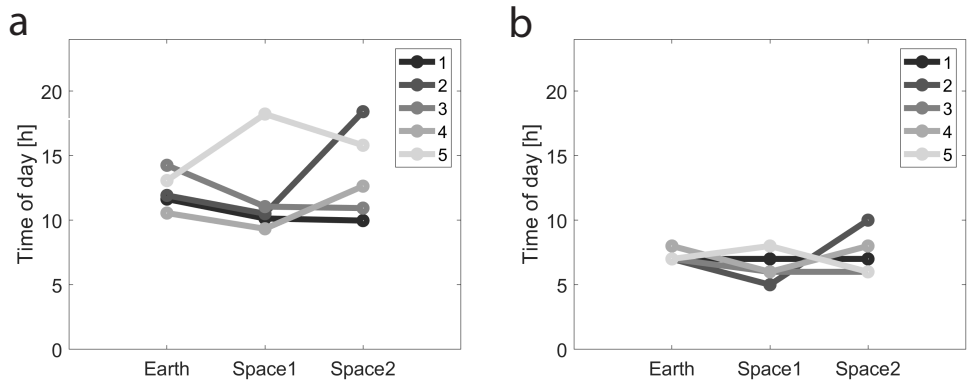

Figure 2.8: Supplementary Figure 1: Three conditions: one on Earth, two in space. (a) For each astronaut, the time of day when the recording was performed was not different across the three conditions: Earth (12.3 ± 0.6), space1 (11.8 ± 1.6) and space2 (13.5 ± 1.6 hours) (linear mixed-effects model with Earth/space1/space2 as a fixed effect and different random intercepts for each astronaut, F(2,12)=0.56, p=0.583, n=15 recording sessions). (b) For each astronaut, the time when they woke up on the day of the recording was not different across the three conditions: Earth (7.2 ± 0.2), space1 (6.4 ± 0.5) and space2 (7.4 ± 0.7 hours) (linear mixed-effects model with Earth/space1/space2 as a fixed effect and different random intercepts for each astronaut, F(2,12)=0.98, p=0.405, n=15 recording sessions). (n=5 astronauts)

Chapter 3

Measures of sleep pressure during isolation at the Concordia station and physical activity as a countermeasure

Electrophysiological measures of sleep pressure during wakefulness in the course of isolation at the Concordia Antarctica station and physical activity as a countermeasure

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Own contribution: *designed the data analysis, negotiated the data sharing agreement, analysed the data, wrote and edited the manuscript*

Publishing status: *BIORXIV/2019/51656*

Abstract

Concordia station in Antarctica is one of the most remote human outpost on Earth. Because of its geographical location, a winterover at Concordia shares a lot of stressors with a space mission. Following a recent study on the markers of sleep pressure during wakefulness on board of the International Space Station, we were investigating if long term isolation in a space analogue station shows similar effects on sleep pressure. Unlike in space, markers of sleep pressure did not increase during isolation's constant darkness period in Antarctica. When measures of sleep pressure were high in the evening, psychological strain was increased, emphasising the importance of keeping sleep pressure under physiological levels during the mission. As a first indication for a countermeasure, we showed that one hour of bicycle exercise during lunch time could decrease sleep pressure. All these observations need to be further studied in a more controlled environment.

Keywords

Sleep pressure, wake electroencephalographic recordings, Antarctica space analogue station, long term isolation, psychological strain, space countermeasure, physical activity

Introduction

Sleep quality and sleep quantity are regulated by two interacting process, the homeostatic and the circadian process [30]: i) The homeostatic process builds up with time awake. Good sleep quality is defined by an efficient dissipation of sleep pressure overnight which in turn decreases the propensity to fall asleep and improve alertness the following day [2, 18]. ii) The circadian process is oscillating on a 24 hours cycle [78]. The master clock is regulated by the suprachiasmatic nucleus and constantly adjusted by environmental cues [139]. Daylight is one of the main cues to the master clock. Circadian rhythms exert a strong impact on sleep quality and sleep timing [92]. Interestingly, EEG theta activity (5-7Hz) in the waking EEG [30] reflects both the homeostatic and the circadian process. The circadian impact can be seen by diurnal fluctuations of theta activity, peaking in the afternoon and again at bed time [232]. The homeostatic component, on the other hand, becomes obvious after prolonged wakefulness (i.e. sleep deprivation), which is associated with a significant increase in theta power. [4, 5, 47, 106, 229].

Astronauts are subject to very irregular schedules and workloads (e.g. launchings or dockings of a vehicule) and while orbiting Earth, the International Space Station (ISS) crew witness a sun rise every 90 minutes which can lead to a misalignment of their 24-hour sleep-wake cycle [109]. Accordingly, chronic sleep restriction has been reported during space missions [15]. We recently provided first evidence for increased of sleep pressure markers during wakefulness in space, i.e measured waking EEG in the theta frequency range, and showed that reaction times were slower when markers of sleep pressure were high [114]. To understand the cause of changes in markers of sleep pressure in space we need to disentangle the effect of each space environment stressor on the human brain.

The Concordia research station shares many stressors with long-term space missions and therefore serves as a useful analogue habitat for research on human physiology and psychology (Fig. 3.1.a). In addition to the harsh environment, Antarctica Concordia station is one of the most remote habitat on Earth, with extreme geographical and social isolation. When temperature drops during the winter (Fig. 3.1.b), air planes cannot reach the station and all participants are in total isolation for eight months. This total isolation particularity adds an additional psychological stress, since the expedition cannot be stopped in case of failure. Moreover, the 80 scientists from the summer crew are reduced to less than 15 during the winterover. Because of its geographical situation, Concordia station crews do not have any daylight for four months during the winter (May-August). Furthermore, the station is at high altitude (3200m) which implies that the crewmembers have to cope with lower oxygen levels (hypoxia) and low air pressure (hypobaria). Because of its location at the South Pole, the atmospheric pressure is equivalent to an altitude of 3800m

outside the polar circle [104]. Since the oxygen concentration is at 13% instead of the usual 21%, the blood saturation in oxygen is lower, which increase the heart rate. Hypoxia has shown to impact cognitive performances and sleep quality [242, 190, 224, 160]. Fortunately, the adaptation process to high altitude takes place within a few weeks and participants should be acclimated to their new environment for their first measurement [104, 184]. Nevertheless, high altitude at Concordia station is a good model of hypoxia and hypobaria conditions encountered during space missions [201].

A complete review of sleep investigations in Antarctica has been recently published [200]. Overall, a sleep quality decrease and a delay in the circadian phase has been reported in Antarctica [200]. However, a high interindividual variability in the acclimatisation to Antarctic environment was reported in all studies. Interestingly, an actigraphy study from both, the coastal Dumont d'Urville station and Concordia station (3200m), showed that sleep disturbances are more pronounced at high altitude and aggravating during the winter [70].

A first aim of this study is to assess long term isolation's effect on measures of sleep pressure using wake EEG data recorded at the Concordia Antarctica research station. The benefit of a good night of sleep is well known and chronic sleep restriction comes with a high cost [162, 95, 247, 171, 170]. Prolonged wakefulness and an increase of theta activity has been associated with an increase of subjective sleepiness, a decrease of alertness and a degradation of cognitive performances [106, 229, 144, 24].

In Antarctica, physical exercise has shown to improve psychological strain in long term isolation [1]. Beside enhancing fitness, strength and compensating for the lack of gravity in space [263], physical exercise countermeasures could also be implemented for cognitive and psychological reasons [36]. Interestingly, a recent study in mice showed that wheel running prolonged the waking bouts of the animals without increasing sleep pressure in the motor and somatosensory areas [108]. Thus, a second aim of this study is to analyse the relation of sleep pressure markers during wakefulness (i.e. theta activity) with subjective psychological strain and to explore physical exercise effects as a potential countermeasure to dissipate sleep pressure during wakefulness.

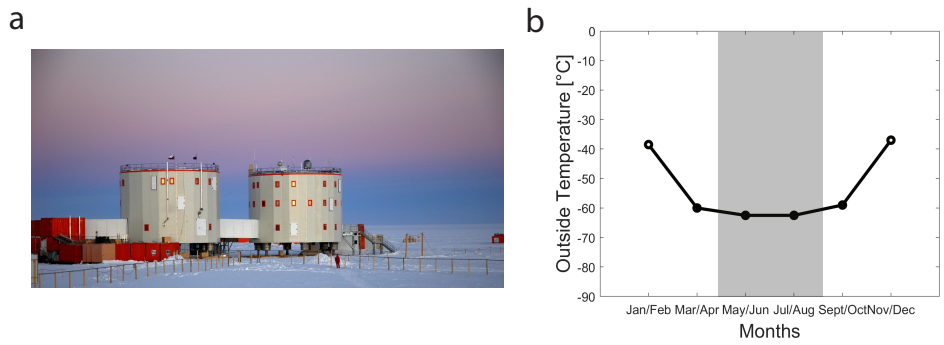

Figure 3.1: Eight months of isolation at the Concordia Antarctica station. (a) Concordia Antarctica station can host 80 researchers during summer time and usually hosts a crew of 15 researchers over the winter. (b) Mean temperature between 2005 and 2015 at the station. Temperature drops during the isolation period. The black dots represent the four measurements used in this study (Mar/Apr, May/Jun, Jul/Aug and Sept/Oct). The gray shadow marks the four months of constant darkness at the station during the winter period, also called the "permanent midnight of winter". (credit: ESA).

Results

Theta power across the day

Because of its circadian and homeostatic component, theta power (5-7Hz) oscillates during the day. Thus, in a first step we tested time of day differences in our recordings. Therefore, we defined two groups; a Noon session with five participants (starting between 10h and 13h) and an Evening session with seven participants (starting between 15h and 20h). When comparing the Noon and the Evening recordings, at electrode C3, we observed an increase in the power spectrum spanning lower frequencies from 2.6 to 7.4 Hz (two sample t-test for each (0.125Hz frequency bin), black dots for uncorrected p-values¡0.05, n=14 recordings at Noon and n=18 recordings in the Evening) (Fig. 3.2.a). We then compared only Theta power at the same electrode. Theta power appears higher at Noon than in the Evening (two sample t-test, t=4.693, p¡0.001, df= 30, n=14 recordings at Noon and n=18 recordings in the Evening) (Fig. 3.2.b).

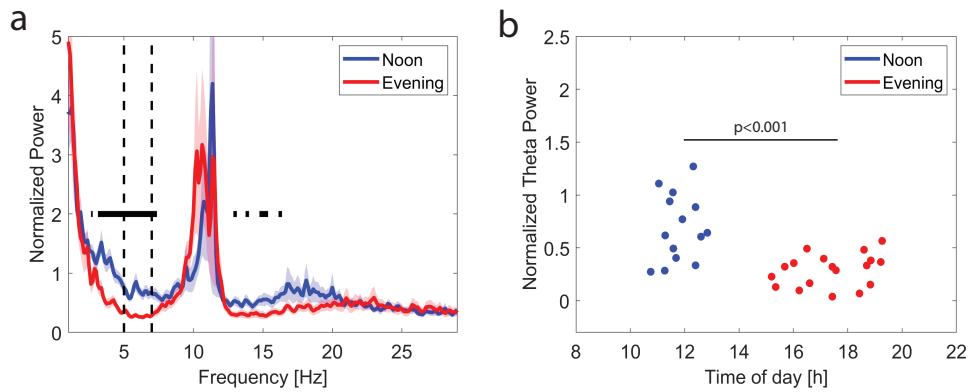

Figure 3.2: Noon and Evening recordings. (a) The power spectrum at electrode C3 for Noon and Evening recordings ($mean \pm sem$). Significant decrease of activity from Noon to Evening in the (2.625-7.375Hz) range (black dots for the uncorrected p-values¡0.05). (b) The blue dots represent theta power at electrode C3 for recordings taken at Noon (n=14 recordings for 5 participants) and the red dots represent recordings taken in the Evening (n=18 recordings for 7 participants). At electrode C3 theta power is higher at Noon than in the evening.

Theta power during isolation period

Each participant was recorded every six weeks during the isolation winter, defining four isolation conditions: Mar/Apr, May/Jun, Jul/Aug and Sept/Oct. We represented the topographical distributions of theta power over the scalp for each isolation condition at Noon and in the Evening (Fig. 3.3.a). Theta power was most pronounced over central and the occipital areas in most of the conditions. When comparing each recording condition to the beginning of the isolation period (Mar/Apr), we found no significant differences in the distribution of theta power during the constant darkness period (May/Jun and Jul/Aug), nor at the end of the isolation period (Sept/Oct) (linear mixed-effects model with the isolation conditions as a fixed effect and different random intercepts for each participant, t-values, n=14 recordings at Noon and n=18 recordings in the Evening) (Fig. 3.3.b).

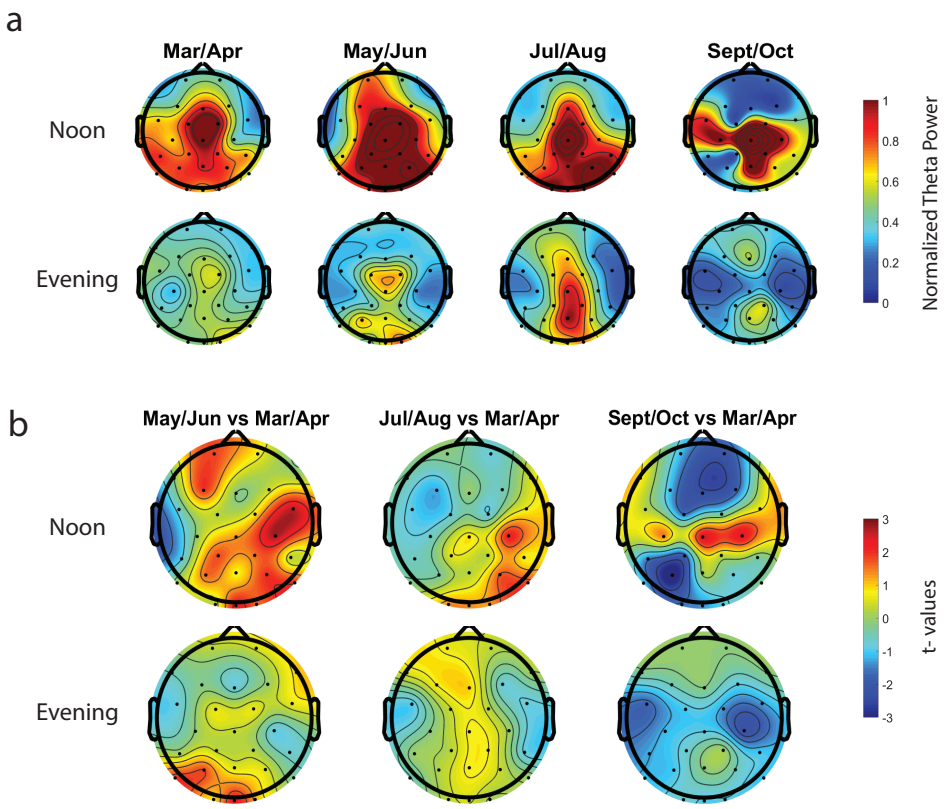

Figure 3.3: Theta power topographical distribution during the isolation period. We have 4 recordings per participant during the isolation period (Mar/Apr, May/Jun, Jul/Aug, Sept/Oct). (a) Normalised theta power per condition for recordings taken at Noon and in the Evening. (b) No significant differences in theta topographical distribution when comparing May/Jun, Jul/Aug and Sept/Oct to Mar/Apr

Theta power, sleepiness and psychological strain

After each resting state EEG recording, the participants had to report their subjective psychological strain by rating adjectives on a questionnaire. We analysed the psychological strain scores' relation with theta power at Noon and in the Evening. We first represented the relation between sleepiness and theta power for all electrodes over the scalp. We found that in the Evening sleepiness was high when theta power across the scalp was high. This was not the case in the Noon recordings. (linear regression model, t-values, white dots for uncorrected p-values¡0.05, for a cluster to be significant it should contain at least two neighbouring electrodes, n=14 recordings at Noon and n=18 recordings in the Evening) (Fig. 3.4.a). Then, while looking at the psychological strain questionnaire's total score, we found that only the right frontal cortex (electrodes F4 and F8), has high theta power when psychological strain scores are high (linear regression model, t-values, white dots for uncorrected p-values¡0.05, for a cluster to be significant it should contain at least two neighbouring electrodes, n=14 recordings at Noon and n=18 recordings in the Evening) (Fig. 3.4.b). For theta power within the right frontal cluster (electrodes F4 and F8), we computed the best linear fit for its relation with the psychological strain scores (linear regression model, $r^2 = 0.363$, p=0.008, n= 18 recordings) (Fig. 3.4.b). This correlation was not driven by any isolation condition in particular and seems to be a general trend.

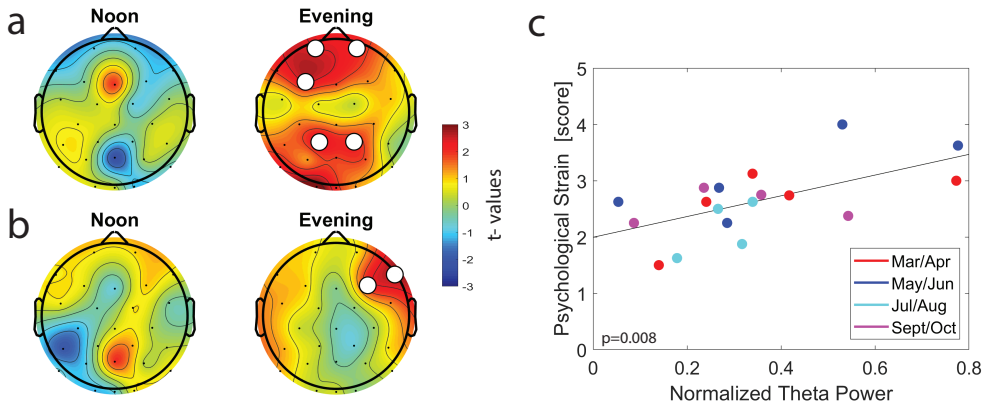

Figure 3.4: Relation between sleepiness, psychological strain and theta power. (a) Topographical representation of sleepiness as a predictor for theta power at Noon and in the Evening. Electrodes in the frontal central and parietal areas (FP1, FP2, F3, CP1, CP2, P7, O1 and O2) have a significant positive slope (white dots for uncorrected p-values¡0.05). Meaning that when theta power in high sleepiness scores are high. (b) Topographical representation of subjective psychological strain as a predictor for theta power at Noon and in the Evening. Electrodes in the right frontal area (F4 and F8) have a significant positive slope (white dots for uncorrected p-values¡0.05). Meaning that when theta power is high psychological strain score are low. (c) Positive slope for the relation between the psychological strain score and the mean theta power at electrodes F4 and F8 for all Evening recordings.

Theta power and physical activity

Then, for approximately one hour ($1.3h \pm 0.1$), each participant had to perform an incremental bicycle exercise. We compared the power spectrum at the electrode C3 Before and After the physical exercise and we found a reduction After the physical exercise in the Noon session in the 3.5-8.5Hz range (paired t-test, blue dots for uncorrected p-values¡0.05, df=13, n= 14 Noon recordings, n=18 Evening recordings) (Fig. 3.5.a). When only comparing theta power (5-7Hz), we found a reduction After the physical exercise in the Noon session (paired t-test, t=-3.487, p=0.004, df=13, n=14 recordings). No such reduction was found in the Evening session (paired t-test, t=0.369, p=0.717, df=17, n=18 recordings) (Fig. 3.5.b). We applied the same approach for each electrode over the scalp and found that theta power was significantly reduced in the central, parietal and temporal areas After the physical exercise in the Noon session (paired t-test, t-values, white dots for uncorrected p-values¡0.05, for a cluster to be significant it should contain at least two neighbouring electrodes, n=14 recordings at Noon and n=18 recordings in the Evening) (Fig. 3.6.b).

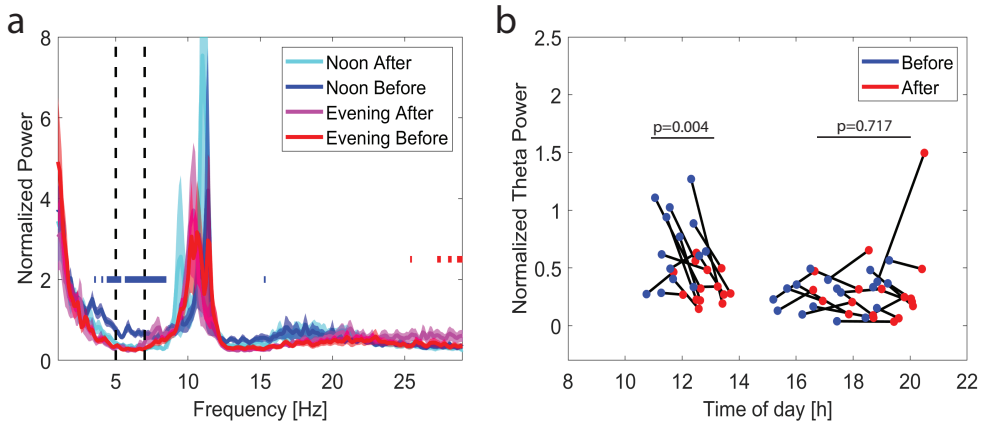

Figure 3.5: Physical exercise effects on theta power. (a) The power spectrum at electrode C3 Before and After the physical exercise for Noon and Evening recordings ($mean \pm sem$). Significant decrease of activity in the (3.5-8.5Hz) range After the physical exercise at Noon (blue dots for Noon and red dots for Evening's uncorrected p-values¡0.05). (b) The blue dots represent theta power at electrode C3 Before the physical exercise and the red dots represent theta power at electrode C3 After the physical exercise. There is a significant decrease of theta power at Noon after the physical exercise. No significant decrease of theta power in the Evening after the physical exercise.

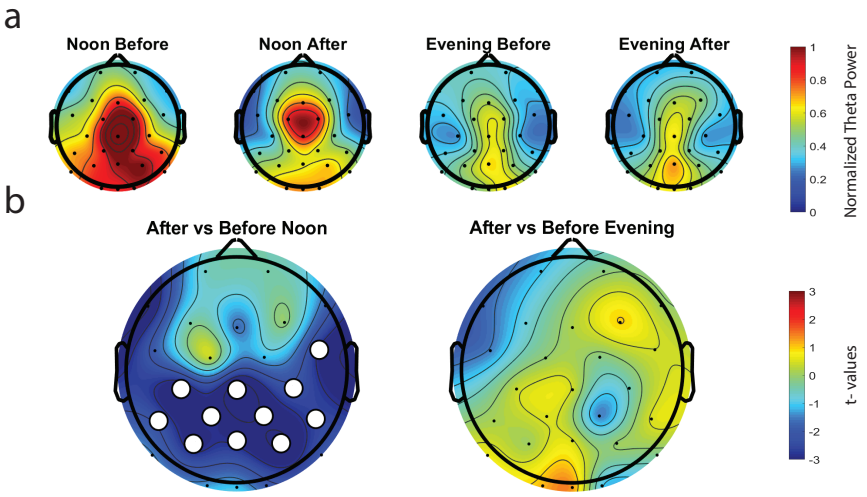

Figure 3.6: Theta power topographical distribution Before and After the physical exercise. (a) Normalised theta power at Noon Before and After the physical exercise and in the Evening Before and After the physical exercise. Consistent central, parietal and occipital distribution of the theta power in all conditions. (b) Decrease of theta power in the central, parietal and temporal areas after the physical exercise at Noon (white dots for the uncorrected p-values¡0.05)

Discussion

We found no differences in theta power across the isolation conditions. However, across conditions, we found that an increase of theta power in the evening in the right frontal area was associated with an increase of psychological strain. Finally, we found evidence that a bicycle exercise is able to reduce theta power, at least at its peak around noon.

Misalignment between the endogenous circadian clock and the sleep wake cycle reduce sleep quality and quantity [163] and chronic sleep restriction is known to increase sleep pressure [170]. In a previous study, we showed an increase of sleep pressure markers in ISS astronauts [114]. One possible explanation for such increased sleep pressure on the ISS might be long-term isolation. However, long-term isolation on Earth at the Concordia station, did not show any influence on electrophysiological measures of sleep pressure. Hence, isolation might not be the cause for increased sleep pressure on ISS. Eight months of isolation at the Concordia station was not sufficient to mimic the six months' space mission on the ISS.

A review by Pagel and Chouker outlined that isolation can impact cognitive performances, stress levels and psychological strain [194]. Our questionnaire was designed to assess psychological strain during the isolation period at the Concordia station. In our study, we showed an increase of subjective sleepiness when theta activity was high in the evening, which confirmed the role of theta power as a sleep pressure marker during wakefulness. Moreover, theta power had no correlation with sleepiness at noon, which confirmed the strong circadian regulation of sleepiness [123]. In the evening, an increase of psychological strain was correlated with an increase of theta power in the right frontal cortex. Usually, theta power is low in the evening, since the circadian component has a strong influence on sleep pressure at this time of day [5, 106, 229]. If the circadian rhythm is misaligned (i.e. phase shift), evening sleep pressure will not properly be compensated for. In turn, theta power would be higher than normal, which could impact sleepiness and psychological strain. The relation between circadian misalignment and mood is well known [28]. However, in our study the correlation between theta power and psychological strain needs to be further explored in a laboratory controlled environment.

Overall, countermeasures need to be implemented to improve the sleep quality and quantity in further interplanetary space exploration missions. Daily physical exercise has shown to help maintain the psychological strain of the participants during the isolation period at Concordia station [1]. Furthermore, Pattyn and colleagues outlined that daily physical exercise can help reduce sleep disturbances in Antarctica [200]. From the oscillation of theta power during the day, we can expect a peak of theta activity at 14h [5, 106, 229]. Accordingly, for recordings around noon, we would expect an increase of theta power after one hour of physical activity. However, in our study, we noticed

a decrease of theta power after the bicycle exercise when the exercise was performed at noon. Our study is a first indication that an ergometer bicycle exercise could decrease sleep pressure in some cortical areas. At noon, the circadian component doesn't compensate for the build up of sleep pressure. It is hence not surprising that only at this time of day we can manipulate sleep pressure with countermeasures. In the evening when the circadian component had a stronger influence on sleep pressure, physical exercise had no effect on sleep pressure markers. To confirm the interest of physical activity as a countermeasure for sleep pressure, further studies would need to record sleep EEG the following night to observe the SWA levels at the beginning of the night and study how SWA dissipates overnight.

In the work by Fisher and colleagues [108] it is hypothesised that wheel running is an automatic movement for mice, also called stereotypic movement. This type of exercise would allow a decrease of firing activity in some cortical areas. In our study, we hypothesise that bicycling in humans could have a similar effect and that an appropriate physical exercise could locally decrease sleep pressure. Even though, physical activity involving novelty and more complex tasks often show an increase sleep pressure [142]. Previous research on the effects of physical activity on cortical activity, revealed that cortical activity was specific to the type of physical exercise, that there is a familiarisation process with the exercise and that there is an intensity response to physical activity [218]. For example, at high intensity and when the subject are familiar with the exercise, a reduction of beta activity was observed in the prefrontal cortex during resting state with closed eyes [39]. During the MARS 500 space analogue mission, cortical activity in the frontal areas was decreased and cortical activity in the parietal lobe was increased after physical activity involving endurance (i.e. two 30 min session of treadmill running and ergometer bicycling) in resting state EEG with closed eyes [217].

Laboratory condition can unfortunately not simulate properly spaceflight conditions. Concordia station shares several psychological and physical aspects of human space exploration and is one of the most remote space analogue facility. Nonetheless, to understanding the mechanisms underlying sleep disturbances in Antarctica and test further sleep pressure countermeasures, we would need a more controlled environment. The station medical doctors, reported night shifts during the winterover for some participants. The main limitation of this study is the lack of information on the sleep-wake history during the isolation period. The amount of sleep the night before the recordings and the wake up time were not reported. Accordingly, we cannot assume that all participants are aligned with their circadian clock. In further experiments, a better controlled sleep-wake schedule should be implemented to study isolation's effect on sleep pressure. In addition to wake EEG data, sleep EEG data and sleep-wake history would have been necessary to confirm our findings. In a previous study, an increase of beta activity in the prefrontal cortex was observed after

an acute hypoxic exposure [219]. Even though a fast adaptation was shown [104], we cannot exclude that hypoxia might affect our results. In this study we had no baseline recordings before participants' trip to Antarctica. As a consequence, we used the recording at the beginning of the isolation period (Mar/Apr) as a baseline to study the other isolation recordings. In Mar/Apr the temperatures are not at their minimum, the summer camp crew just left the station and the winter crew is preparing for the winterover. Similarly, the Sept/Oct isolation recordings represents the end of the isolation period, since the sun rise again and planes start coming back to the station. For each participant and along the winterover, experiments were performed at different times during the day. To compare recordings within participants, we had to exclude about 1/4 of the recording sessions when recorded at $\pm 1 hour$ from the other recordings. Because of this exclusion criteria we used a mixed effects model to compensate for missing recordings. In EEG studies, it is common to observe inter-individual differences between participants [107]. Hence, it is not ideal to compare Noon recordings with Evening recordings with different participants in each group. Moreover, participants were recorded over two years (DC7 and DC8), which could increase inter-individual differences.

Taken together, our results suggest that increased sleep pressure in the evening impacts psychological strain and physical activity at noon could be envisioned as a countermeasure for high sleep pressure.

Methods

Participants and experiment

12 participants (all male, 40 ± 0 years old) took part in the experiment (ESA Experiment record n° 9363) over the winter 2011 and 2012 (winterovers DC07 and DC08) at the French-Italian Concordia research station in Antarctica (Fig. 3.1.b). Written informed consents were obtained prior to participation. The experimental protocol was approved by the European Space Agency's Medical Board (ESA-MB) and the ethic committee from the Institut Polaire Paul Emile Victor (IPEV). During the period of isolation, the participants took part in the experiment once every six weeks. During each recording session, the participants had a five minutes resting state EEG recording with eyes open and they had to answer to a psychological strain questionnaire. This was followed by an incremental bicycle exercise lasting 1.3 ± 0.1 hour for each session. Finally, a second five minutes resting state EEG, with eyes open, was recorded. Also 5 minutes of wake resting EEG with eyes closed was recorded just after each open eyes recordings. We decided to only analyse the open eyes data since, theta power during closed eyes doesn't correlate with sleepiness [150]. In this study, we defined the recording conditions for each session during the isolation period with the labels Mar/Apr, May/Jun, Jul/Aug and

Sept/Oct. A training session to get familiar with the tasks was performed at the Concordia station three weeks before the beginning of the winter period (i.e. winter between Mar/Oct). However, these recordings were not used in this analysis. Out of the 48 recording sessions, four sessions were not well recorded and excluded from the dataset. Recordings took place at variable times along the day. We excluded all sessions performed at more than ± 1 hour from the median time of recording throughout Mar/Apr, May/Jun, Jul/Aug and Sept/Oct sessions for each participant. Out of 44 sessions, 12 sessions were excluded from further analysis because of this lack of circadian similarity between the isolation sessions. During the winter's constant darkness period (i.e. May/Aug), the temperature drops rapidly toward a mean temperature of -60° with peaks at -80°C (Fig. 3.1.a).

Wake EEG recordings

Each participant, for each session, was recorded with a 32 channels actiCAP (BrainProducts, Gilching, Germany) (10–20 electrode system EEG cap). Continuous wake EEG with eyes open was recorded for 5 minutes during each session at a sampling rate of 500 Hz and down sampled to 250Hz. Scalp electrodes' impedance were measured and kept below 10 KΩ. All channels were referenced to FCz. ECG and electrodes TP9 PO9 Po10 T7 and T8 were excluded from further analysis to standardise DC07 and DC08 recordings. Out of the 32 electrodes recorded, 26 remained in our analysis. EEG data pre-processing was performed in Matlab (Version R2018b) using EEGLAB toolbox scripts (Version 14) [86] and additional custom made scripts. EEG data were down sampled to 250 Hz and pass-band filtered [0.1–48 Hz]. Each EEG channel was referenced to the average activity across the channels (i.e. average referencing). An Independent Component Analysis [87] was performed to remove ocular, muscular, and electrocardiographic artifacts (3.12 ± 0.18 components rejected per recording) as defined by Hulse and colleagues [143]. Using the EEGLAB graphical user interface, all movement's artefacts in the signal were marked by visual inspection and removed. The power spectrum was computed for each channel and outliers containing high muscle artefacts (20-30Hz) were excluded from the dataset (1.89 ± 0.15 channels rejected per recording) [102]. For each subject, rejected channels were interpolated. To reduce the signal to noise ratio, we performed a phase-rectified signal averaging (PRSA) [161]. PRSA allows to superimpose the oscillations to create an interference and hence reduce the weight of acute noise generators in the signal. The power spectral density was estimated using the Welch's averaged periodograms with a four second Hamming window and a frequency resolution of 0.125 Hz. In each frequency bin, the power at each channel was normalised by the average power over the scalp. The theta power band was computed between 5 and 7 Hz.

Psychological strain questionnaire

The participants completed a questionnaire measuring their psychological strain. This questionnaire is derived from the "Eigenzustandsskala" (EZ-K, which is translated as "Personal state scale") [188]. The questionnaire measured the subjective psychological strain by asking the participants to rate their sleepiness, psychological strain, calmness and recovery state between zero and five. In our study, we reported the psychological strain questionnaire score, rated between zero and five. Since the questionnaire had one question about sleepiness we extracted this unique variable and reported the score, also rated between zero and five. Five being the highest level of sleepiness and zero being the lowest. The questionnaire was presented in the participants' native language.

Physical exercise

The particpant had to perform an incremental bicycle ergometer exercise. The physical exercise started with a 50 Watts workload and increased by 30 Watts every 3 minutes until subjective exhaustion of the participant (lasting 1.3 ± 0.1 hour for each session).

Statistics

In all statistical analysis we first assessed normal distribution of the data with a quantile-quantile plot. When comparing measures in different participants, for example when comparing the Noon and the Evening sessions, we performed a two sample t-test (t=t-value, p=p-value, df=degrees of freedom). When comparing measures within participants in two different conditions, for example when comparing theta power Before and After the physical exercise, we performed a paired t-test (t=t-values, p=p-value, df=degrees of freedom). When studying repeated measures within the same participants, for example when comparing isolation conditions, we performed a linear mixed effects analysis for the relationship between the response variable and the fixed/random effects. We used the restricted maximum likelihood estimate method to fit the model and choose the best model based on Bayesian information criterion results. Visual inspection by quantile-quantile plot of the residuals confirmed that homoscedasticity and normality were respected. Then, we completed a two-sided t-test (t=t-value, p=p-value, df=degrees of freedom) with the null hypothesis that each fixed effect in the model has a coefficient of zero. When the best model to study the response variable contain only one fixed effect without random effects, for example when looking at the relation between theta power and psychological strain scores, we used a linear regression model to obtain a R^2 value which indicates how much of the total variation can be explained by the fixed effect. Then, we completed a two-sided t-test (t=t-value, p=p-value, df=degrees of freedom) with the null hypothesis that the slope of the model is equal to zero. For the topographical representations we plotted the t-values for the aforementioned t-tests and we draw white dots for significant p-values (uncorrected p-values¡0.05). Then, we completed a non-parametric permutation test (critical value=2.14 at Noon or 2.10 in the

Evening, 500 permutations, n=14 recordings at Noon session and n=18 in the Evening) for multiple testing correction [142, 101], defining the minimum cluster size for a pattern to be significant. In our analysis at least 2 neighbouring electrodes needed to be significant to be reported as a significant effect. All statistical analysis were performed in Matlab.

Data availability

All relevant data will be available from the corresponding authors upon request and after approval from the European space Agency Medical Board (ESA-MB).

Code availability

All relevant scripts will be available from the corresponding authors upon request.

Acknowledgements

This work was supported by the research grant 50WB0819 from the German Space Agency (DLR) and by a research grant from the University of Zurich (Clinical Research Priority Program Sleep and Health). It was also supported by the European Space Agency (ESA) and the Luxembourg Government.

Author contributions

V. Abeln and S. Schneider conceived the experiment. G. Petit, R. Huber and L. Summerer designed the data analysis study. G. Petit performed the data analysis and wrote the manuscript. R. Huber gave data analysis support. All authors contributed to the scientific discussion and manuscript revisions.

Competing Interest

The authors declare that they have no competing interests.

Chapter 4

Hibernation and torpor, prospects for human spaceflight?

Hibernation and torpor, prospects for human spaceflight?

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Own contribution: *Answered invitation to write a chapter for the handbook, wrote the abstract/introduction/conclusion and defined structure of the chapter, invited the ESA topical team on hibernation members to contribute in the project regarding their own expertise, harmonised the writing materials, edited and published the manuscript.*

Publishing status: *Springer, as part of the Handbook of Life Support Systems for Spacecraft and Extraterrestrial Habitats:1–15, (September 2018).*

Abstract

Despite substantial technical progress in the last decades, long-distance human space travel beyond the Earth-Moon system still represents a challenge. Resources for sustaining humans in good physical and mental health on such journeys are important drivers of complexity and cost. Every kilogram of mass and every cubic meter of volume reduction will reduce the total cost and increase the feasibility and thus the chances of success of such missions. Torpor and hibernation are solutions developed by animals to cope with severe resource restrictions and hostile environments. Reducing the demands on life support systems by putting humans into a state of hibernation might appear like a story straight out of science fiction. This chapter attempts to demonstrate that this concept is no longer in the realm of science fiction by providing an overview of the current scientific understanding of the processes of hibernation and torpor.

Keywords

Endothermic animals, Synthetic Torpor, Artificial Torpor, Torpor, Hibernation, Hypometabolism, Basal Metabolic Rate, Metabolic depression, Homeostasis, Sleep, Thermoregulation, Ionizing radiation, Radioprotection, Microgravity, Muscle atrophy, Oxidative stress, Cellular mechanisms, Neuroscience, Human space physiology, Human space medecine, Microgravity analogs, Space countermeasures

Introduction

Various animal species exhibit bouts of reduced metabolism, most of the time along with decreased body temperature (T_b), either on a daily basis or for weeks at a time, also called *torpor*. Recurring long-lasting bouts of torpor, as achieved by active metabolic suppression, are known as *hibernation*. Strategies such as torpor and hibernation have evolved to reduce energy expenditure to help animals to cope with periods of scarce resources and adverse environmental conditions. Endotherms , animals that regulate their body temperature by harnessing internal heat sources (e.g. mammals), are less susceptible to cold, but need to maintain a constant core temperature for optimal enzyme activity. This thermoregulation capacity of endotherms leads to an energy consumption which is much higher than those of their ectothermic counterparts (e.g. reptiles, fish). The latter have negligible internal thermoregulation and can endure core temperatures fluctuating within a wide range of ambient temperatures (T_a). The unfavourable scaling of surface-to-volume ratio and the resulting higher heat loss, led especially small endotherms to adopt other

strategies such as torpor and hibernation to cope with low temperatures and limited energy intake [117].

In adult humans the basal metabolic rate (BMR) is defined as the minimum energy requirements per unit of time in a thermoneutral environment when inactive, not during gestation or after ingesting aliments [174]. Certain hibernators can reduce their metabolism by 95% compared to their basal metabolic rate [228]. The ability to safely reduce the average metabolic rate in astronauts during long-distance space exploration missions would result in a number of advantages and potentially address a range of currently critical open questions.

In a 950 days mission to Mars with six crew members, alongside extensive life support systems, consumables at launch (e.g. oxygen, water, dry food) would represent a mass of about 10 tonnes [230]. During a 14 months human mission from Earth to Mars and back, consumables would approximately account for a mass of 4-5 tonnes. An astronaut BMR reduction of 75%, similar to those observed in large hibernators such as certain bears [240] would therefore result in substantial mass and cost benefits, eventually being even mission enabling.

Inducing hypo-metabolic states in astronauts would impact the size of the transfer habitat as less volume would be required for human activities. Furthermore, psychological stress due to confinement and a monotonous routine could be alleviated by torpor. Due to the diminished payload and life support systems requirements, less energy would be needed. Various protective features of hypo-metabolic states could conceivably simplify radiation shielding and thermal protection design, and prevent muscle and bone atrophy. [58, 119].

The following sections are addressing some key aspects of hibernation and torpor: The first section shows the diverse occurrences and forms of torpor and hibernation as a resource saving mechanism in mammals which suggest that the basic mechanisms might be present in all mammals including humans. The second section explores the current understanding of the relation of torpor and hibernation to sleep, brain functions and homeostasis. The following sections then explore mechanisms to induce such states including thermo-regulation and cellular level pharmacological pathways. The last section sheds light on the current understanding of the mechanisms to explain why torpor and hibernation seem to offer protective aspects against some of the critical factors for long-duration human spaceflight such as bone and muscle loss, and exposure to high levels of ionizing radiation.

The diversity of mammalian torpor behavior

Mammals are endothermic and enjoy the advantage of a constant high body temperature (T_b) at 37°C. Their resulting high energy expenditure requires a constant food supply. Long-term shortages of food supply may occur in

many habitats, including northern, boreal, arid or even tropical climatic zones. Torpor is the most powerful and flexible physiological mechanism to cope with limited resources.

Torpor is characterized by a temporary reduction of metabolic rate, heart rate, ventilation, and cellular processes, as well as the down-regulation of T_b approaching the level of ambient temperature (T_a). Metabolic rate may be lowered to a fraction of basal metabolic rate [134, 215]. If this occurs as short torpor bouts for several hours per day, it is called daily torpor. During hibernation torpor bouts are longer than 24 hours, i.e. multi-day torpor bouts, typically lasting 7-20 days. Hibernation is a sequence of such multi-day torpor bouts interrupted by 1 or 2 days of arousal (interbout arousal). Daily torpor may save up to 70% of energy expenses (e.g. Djungarian hamsters). Hibernation is even more efficient by saving up to 95% (e.g. marmots) over a prolonged period of time including the extra energy required for interbout arousals.

Arctic ground squirrels (*Urocitellus parryii*) hibernate for about 8 months in underground burrows surrounded by frozen soil. Their T_b may drop as low as -2.9°C which is below the freezing point of body fluids [16]. This super-cooled state of body fluids is maintained despite continued ventilation movements, heart beats, and thermo-regulatory heat production in deep torpor [212]. These are the lowest body temperatures known for endothermic mammals. Most hibernators try to avoid T_b below the freezing point of body fluids by selection of appropriate micro-environments in caves (bats) and counteractive thermo-regulatory heat production in the torpid state.

Alpine marmots (*Marmota marmota*) also hibernate for about 7 months at soil temperatures decreasing below the freezing point during hibernation, but they maintain their T_b well above the freezing point at about 2-4°C [10, 11]. The maintenance of a high T_b in hibernating marmots is facilitated by hibernation in large social groups and a remarkable potential for thermo-regulatory heat production in the torpid state.

The common dormouse (*Glis glis*) can be considered as one of the record holders for its hibernation season duration, lasting up to 9 months when in the fields. Dormice kept in aviaries with underground burrow systems stay in hibernation season for up to 19 months, skipping the summer active and reproduction season until the next year [105, 26].

Hibernation is not limited to cold environments but may also occur in warm environments. Fat tailed lemurs (*Cheirogaleus medius*) inhabit tropical rain forests on Madagascar. Prior to the dry season (i.e. March to September in the Southern hemisphere), they accumulate fat largely in the tail and then retreat into tree holes for hibernation. They reduce their metabolic rate by 70% compared to their normal active state and their T_b drops to values close to T_a. T_a in the tree hole may drop to 15°C at night and may rise to about

35°C during daytime. As a consequence T_b oscillates between 15 and 34°C. Despite this large fluctuation and T_b close to their active state, lemurs remain torpid with a depressed metabolism [80, 81].

American black bears and brown bears are further examples for hibernation at high body temperature above 30°C [240, 100]. Their torpid metabolic rate is as low as expected for a mammal of this size but due to their large body mass and the well insulating fur, T_b remains close to normothermia [240]. It is of interest to note in the context of discussing the potential operational capabilities of astronauts, that female bears can be pregnant during hibernation, give birth, lactate and nourish the offspring solely from their body fat stores.

Daily torpor is less effective for long-term energy savings. However, it offers a greater flexibility in response to shortage of resources. Hibernation needs several weeks of preparation, including the accumulation of body fat, the preparation of an appropriate hibernaculum, and even long distance migration to wintering sites in the case of bats [258]. Once preparations are completed, most hibernators continue hibernation until the following spring. Daily torpor in contrast can be adjusted day by day to the availability of resources. This is most obvious in mice which enter torpor when transferred from thermo-neutrality (30°C) to cold environments (20°C) and food is removed simultaneously [257, 94, 191]. In Djungarian hamsters (*Phodopus sungorus*), daily torpor is part of seasonal acclimation and occurs spontaneously at thermo-neutrality and food *ad libitum* during the winter season (i.e. short photoperiod), but moderate cold or food shortage may enhance the incidence and depth of torpor [135].

The widespread occurrence of torpor behavior in the mammalian tree suggests that basic properties of torpor physiology are to some extent available in most mammals. Lemurs are using daily torpor and hibernation in a rather regular manner which provokes the speculation that other primates, like humans, also have the physiological tools for entering torpor. Several candidate hormones and metabolites for metabolic depression have been tested to induce torpor. Some effects have been observed but none of the treatments induced the full spectrum of physiological responses observed in natural torpor [32].

Torpor, sleep and homeostasis

In most organisms living on Earth, the 24 hour daily cycle has a profound impact on behavioral, physiological and metabolic processes, which are essential for survival, especially under conditions of stress [22, 30]. In mammals and many other species, sleep and energy metabolism are intimately linked. This is evidenced by the numerous bidirectional connections between the neural centres that govern these processes [3, 8]. Distinct neuronal populations within the ventromedial, dorsomedial and arcuate nuclei of the hypothalamus

respond to and integrate peripheral energy signals (e.g. glucose, fatty acids, leptin, ghrelin, corticosterone, etc) to dictate appropriate feeding and energetic responses to peripheral energy status [42, 223].

Critically, all of these homeostatic centres are also implicated in regulating sleep physiology, or project to sleep regulatory and arousal promoting pathways, and are also known to be central to the expression of torpor. Torpor is a unique adaptation to harsh environmental conditions, characterized by a profound attenuation of physiological functions, including the drop of body temperature to within a few degrees of ambient temperature [155, 96].

The expression of torpor is a strictly regulated process, and one that is readily reversible and without lasting consequences to the animal. As such, induction of torpor-like hypothermic states has been gaining increasing attention for use in clinical settings and space applications. However, many of the mechanisms that regulate this dramatic physiological state remain poorly understood.

The nature of the relationship between torpor and sleep has been a controversial topic for several decades. Animals typically enter torpor through sleep, and animals in torpor appear to be sleeping, yet it is unclear whether torpor is a state neurophysiologically and/or functionally similar to sleep, or is fundamentally different [154]. For example, at moderate ambient temperatures, hibernation in golden-mantled ground squirrels resembles a virtually continuous electrophysiological state of sleep, which is essentially isomorphic with slow wave sleep episodes at non-hibernating body temperatures [255]. Sleep is known to be important in cognitive function , memory, and synaptic plasticity; it has also been proposed that sleep plays an important functional role in metabolic regulation [178, 89, 66, 252]. Furthermore, increasing evidence suggests that sleep deprivation represents a major challenge for energy homeostasis , and is associated with changes in glucose tolerance and insulin resistance in both humans and laboratory animals [147, 245]. However, while sleep and torpor are closely linked, the nature of their relationship is still not fully elucidated.

The humans of waking and sleep is regulated by several subcortical structures, which provide neuromodulatory action on the forebrain [216]. In addition, wakefulness and sleep are shaped by the interaction of two processes: the sleep-wake dependent process, which keeps track of time spent awake and asleep and the circadian process, which provides a temporal framework for specific waking behaviors, sleep and metabolism [30, 21]. Sleep loss is compensated by a subsequent increase in sleep intensity, manifested in the levels of cortical electroencephalographic (EEG) slow-wave activity (SWA, 0.5–4.0 Hz) in non-rapid eye movement (NREM) sleep [252]. Notably, emergence from both hibernation and daily torpor in seasonally hibernating species is associated with a substantial increase in EEG slow-wave activity [84, 79], a hallmark of deep restorative sleep, typically encountered after sleep depriva-

tion [79]. Several studies have shown that the entry into torpor is associated with a substantial loss of synapses in several brain regions and altered dendritic morphology, which are restored within two hours after emergence from torpor [116, 214, 207]. Therefore, while the functional role of deep sleep after torpor is yet unclear, one possibility is that sleep is important for recovery processes, which could be related to energy homeostasis, synaptic structure and function, and ultimately for a renormalization of learning and waking performance [116, 214, 207].

The factors involved in the induction and maintenance of spontaneous torpor and the effects of torpor on brain activity remain elusive. Elucidating neural mechanisms of torpor and clarifying the relationship between torpor and sleep will benefit numerous clinical applications, and will open new frontiers for investigating the possibility of human hibernation for deep space exploration.

Thermo-regulation

Dropping body temperatures are often observed during torpor and hibernation, though as demonstrated by normothermic hibernators, it is not necessary. In mammals, thermo-regulation is the combination of autonomic and behavioral responses that adapt heat production and heat dissipation to the body's needs, independently from the ambient temperature. Thermo-regulation is often explained by referring to the theory of setpoint [261]. According to setpoint theory, the brain senses and regulates body temperature in alignment with a set temperature. This theory has been challenged [35], and an open loop theory has been proposed in substitution [213]. The open loop theory rejects the idea of one controller regulating a unified system, suggesting that each thermo-effector (i.e. shivering, sweating, ...) acts independently from the others, and is turned on or off by its neural controller at different temperature thresholds. Body temperature , therefore, is understood as the result of the parallel activity of all these open loops [173].

If entrance into torpor or hibernation could be triggered by passing a physical setpoint, a mimicking condition, sometimes referred to as artificial or synthetic torpor [56], could be induced in other non-hibernating animals by decoding how temperature is encoded in the neural setpoint and by changing such values.

Cellular mechanisms of hypometabolism and pharmacological interventions

Metabolism is commonly assessed by measuring oxygen consumption. Mitochondria are cell organelles that consume the majority of oxygen (90%) while transforming sugar and fats to generate ATP or simply heat. Given the large

drop in oxygen consumption at torpor entrance, active metabolic suppression likely involves inhibition of mitochondria. Changes in mitochondrial function of hibernators have been mostly explored in isolated mitochondria fuelled by specific substrates, which in essence explores its (maximal) capacity, rather than its explicit functioning in the animal. Moreover, arriving at an overall conclusion is hampered because of important differences between studies in substrates, temperatures, organs, species and seasonal timing (for latest review see [226]). Generally, changes in mitochondrial respiration seem to precede hibernation [133] and suppression of respiration in mitochondria isolated from torpid tissue seems early [64] and modest [133] in the abundance of substrates – if at all present in specific tissue such as brain [13, 115]. Consequently, the reduction of mitochondrial respiration at entrance of torpor cannot be (totally) accounted for by a decrease in mitochondrial capacity to oxidize substrates. Therefore, additional mechanisms conferring inhibition of mitochondrial function are thought to encompass rapid and reversible modifications of key proteins of mitochondrial respiration and/or transport of its substrates, such as phosphorylation, acetylation and nitrosylation. Moreover, recent research results suggest that mitochondria and mitochondrial quality control mechanisms differ fundamentally between cells from hibernators and non-hibernators [137].

The advent of 'omics' techniques has recently allowed for an unbiased exploration of changes in gene expression and protein abundance during different phases of hibernation. No unique genes have been identified in hibernating species and genes differentially expressed during hibernation do not differ in a specific manner from non-hibernator genes [250]. The main changes in expression involve genes that confer the metabolic adaptation from glucose towards lipid combustion (β-oxidation) and those that harness anti-oxidant defence [248]. The few proteomic analyses confirm the abundance of proteins involved in fatty acid oxidation during the hibernating season and identified specific proteins of interest in distinct organs [9, 126]. However, no substantial shifts in gene or protein expression have been documented at torpor entrance.

Studies exploring mitochondrial function and 'omics' indicate that the most obvious changes occur from summer to just prior to or after initiation of hibernation. This matches the notion that hibernating animals cannot initiate torpor overnight, but generally need a preparatory phase as reflected e.g. in their fattening up or adaptation of fur coat. There is emerging evidence that changes in gene expression underlying the preparation for hibernation are governed by epigenetics [7], i.e. specific chemical changes to DNA bases or to histones, the proteins that package DNA. By acetylation and methylation reactions, epigenetics thus dynamically orchestrates silencing or expression of sets of genes in response to circannual, circadian, seasonal and photoperiodic rhythms in hibernating species.

Because of the large and fast changes in metabolic rate at torpor entrance or exit, hibernators face the injurious effects of repetitive oxidative stress due to hypothermia, hypoperfusion and/or reperfusion upon arousal. However, animals do not show signs of organ damage during arousals and do not suffer from atrophy of bone, muscle and intestine by disuse. Likely, this seems rooted in mobilizing defence mechanisms by augmenting Nrf-2 related antioxidant pathways [187, 192], suppression of the immune response [31] and blood clotting [82], which may be intimately connected to changes in mitochondrial function.

Collectively, these data imply that the hibernation phenotype is enabled by a selective adaptation of gene expression during a preparatory phase, which equips the animal to experience recurrent torpor bouts. However, the nature of the instantaneous inhibition of mitochondrial respiration at initiation of torpor is still elusive.

Given the current state of knowledge, could a (pharmacological) strategy to induce torpor in humans and thus in astronauts already be defined? In addition to mitochondrial inhibition, physiological and molecular studies have identified several metabolic and temperature controlling pathways that show important changes across the hibernation cycle. These comprise neuro-endocrine pathways involving hypothalamus, brainstem and the sympathetic nervous system. Different signalling molecules and hormones have thus been implicated, including neuropeptide Y, adenosine/AMP, hydrogen sulphide (H_2S) and thyroid hormones [reviewed in 32]. Administration of these compounds has subsequently been shown to induce a hibernation-like state , particularly in small animals [27, 38, 145]. Likewise, pharmacological manipulation of central temperature controlling pathways precipitates a similar effect in rat [57]. Based on the finding of cellular protection by H_2S in hibernation [234, 233], a class of compounds has been developed that harnesses mitochondrial function [131] and protects from deep cooling induced damage [129, 251].

Collectively, these compounds offer important elements for metabolic inhibition and cellular protection . Likely, such strategy is rooted in a versatile way to induce mitochondrial inhibition, either directly, or via activation of specific cellular pathways. Currently planned experiments will investigate whether usage of these compounds induces torpor in a large animal, such as pig, and address questions related to the usage of hypothermia, the need and timing of arousals, safety aspects, and preservation of muscle and bone mass. Specific preparatory measures may be needed, perhaps including manipulation of ambient temperature, light exposure and/or food intake to trigger cellular adaptations via epigenetics . Further studies are needed to refine current knowledge and disclose additional permissive or protective factors enabling torpor induction. To this end, molecular changes in hibernators should be explored by integrating metabolic, physiological and 'omics' data obtained at high sampling frequency, particularly around the initiation of spontaneous

torpor.

Protective aspects

The repetitive, long bouts of torpor during hibernation result in a prolonged (months) period of inactivity with, surprisingly, no muscle atrophy nor decrease in bone density . The mechanisms that preserves the functionality of the musculo-skeletal system are not yet understood. An active change in protein transcription dynamics (e.g. reduction of transcription and degradation), could be at the root of it, possibly preventing proteolysis [59, 248, 9].

Another interesting feature of torpor, along with the preservation of muscle strength and bone structure, is radioprotection. Ionizing radiation from the sun and from interstellar space represent one of the major hazards to the health of astronauts during long spaceflights. Passive shielding and reduction of exposure duration are currently the predominant approaches to protect astronauts, putting substantial constraints on space mission designers [98].

Studies on the resistance of hibernators to very high levels of X- and γ-rays a few decades ago have shown that hibernation enhances radioprotection [58]. Whether induced torpor or torpor-like states in non-hibernators [57, 243] would yield similar protective effects is yet unknown. Furthermore, experimental data are still missing on whether the protective effects measured in X- and γ-rays could be extended to the spectrum of ionizing radiation encountered in space.

The mechanisms underlying radioprotection associated with hibernation are to be found in the interaction between radiation and the metabolic adaptations of the cell to torpor: the halting of the cell cycle and the hypoxia at a tissue level are among the plausible explanations. Since the protective effects seem to take place even when torpor is induced after irradiation, additional mechanisms might be involved [121]. Potentially DNA-repair systems may be more effective when working at a low temperature, as it has also been suggested by *in vitro* data [12].

While extensive experiments have not yet been performed, current technical capabilities would allow the experimental assessment of torpor and hibernation in counteracting the effects of exposure to ionising radiation and micro-gravity in space. Ground based ionising irradiation facilities could validate the experimental protocols, e.g. by comparing the effects of constant radiation exposure in hibernators to those of a torpor restricted group, or by comparing those effects in well-fed mice to mice in which daily torpor-like bouts would be induced by fasting.

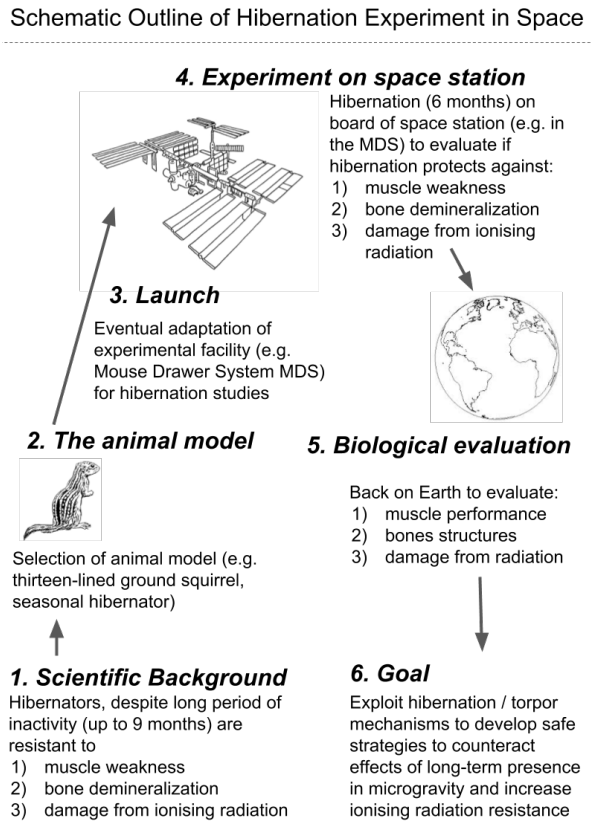

Figure 4.1: Schematic view of potential hibernation experiment on a space station.

Unfortunately, micro-gravity research in animal models, offers only few analogues. Neither hindlimb suspension protocol, nor parabolic flights studies would be appropriate for studying long-term effects of micro-gravity on hibernators. Hypergravity experiments (e.g. centrifuge) could address some aspects of gravity-related effects at the cellular level. However, as illustrated in Fig. 4.1, exploring physiological changes occurring in micro-gravity (e.g. bone and muscle loss) would need to be conducted directly on board space stations. The current simplification of procedures to conduct experiments on board of the International Space Station and the construction of a Chinese space station are increasing the opportunities for such exploratory experiments in micro-gravity.

Conclusions

Based on recent progress in understanding the nature and the underlying mechanisms of torpor and hibernation, these natural solutions to cope with severe resource restrictions and hostile environments have confirmed their attractiveness for human spaceflight beyond Earth orbits. While many aspects of torpor and hibernation are still not fully understood, scientific evidence show that techniques for inducing torpor-like states in non-hibernators could benefit not only deep space exploration but also a large number of medical conditions such as stroke, cardiac failures, complex surgery or transplantation.

Chapter 5

Discussion

Overall findings The aim of this work was to study sleep pressure on the ISS and during space analogue missions. We investigated the implication of a high sleep pressure on behavioural markers and we explored possible countermeasures. First, we studied sleep pressure markers on ISS astronauts during their mission. We found that electrophysiological and cognitive performances indicated an increase of sleep pressure in space. Second, we studied sleep pressure markers at the Concordia station. We found that sleep pressure was not increased during the isolation period. However, high sleep pressure was linked with psychological strain. In addition, we showed that physical activity could be envisioned as a countermeasure. Third, we suggested hibernation-like state as an enabler for manned missions beyond Earth orbit.

5.1 Evidence for increased sleep pressure in space

Findings Our analyses confirmed the negative effects of the ISS environment on sleep and provided first evidence for increased sleep pressure in space [114]. We observed a global increase of theta power in space and we showed that local sleep-like events are more widespread over the scalp (i.e. increased globality) in space compared to Earth. In addition, we reported an increase of slow reaction times after two months in space and reaction times were correlated with the globality of the local sleep-like events. In Antarctica, we found that high sleep pressure in the evening was correlated with psychological strain [113]. However, the performances of the astronauts in a visuomotor task were not associated with local sleep-like events [114].

5.1.1 Implication for astronauts' performances

To improve spaceflight missions' success rate and to decrease the psychological stress of the astronauts, it might be crucial to keep sleep pressure to a physiological level. We showed that reaction times in a visuomotor task

were associated with higher sleep pressure markers. Despite alertness, high sleep pressure might impact many other behavioural outcomes. Nevertheless, we showed that more complex tasks, like docking a Soyuz capsule, were not impacted by high sleep pressure.

The Epworth Sleepiness Scale questionnaire and the Karolinska Sleepiness Scale questionnaire are frequently used to study subjective daytime sleepiness. The questionnaires scales correlate with an increase of theta power during eyes open [150]. However, subjective sleepiness questionnaires cannot substitute a quantitative performance test. Sleep questionnaires have shown to underestimate sleep quantity when participant had 8 hours of sleep opportunity. In contrast, sleep quantity was overestimated when sleep restricted and sleep deprived [169]. The psychomotor vigilance task (PVT), developed by Dinges and colleagues, measure alertness[95]. The sleepiness questionnaires correlate with an increase of PVT reaction times [150]. The propensity to fall asleep is regulated by sleep pressure [2]. Daytime sleepiness is defined by sleep propensity and can be quantified by the multiple sleep latency test (MSLT) [53], which is commonly used to diagnose hypersomnia and obstructive sleep apnea. To define sleep latency, experimenters need to score the first epoch of sleepiness. Hence, MSLT often involves EEG recordings in the process. In sleep deprivation studies, theta power is increased and sleep latency is decreased [47].

Working on cognitively demanding and sustained tasks lead to mental fatigue [246]. There is a major difference between sleepiness and fatigue, even though sustained cognitive activity will also lead to sleepiness with the time awake. Sleepiness impacts alertness [247], while mental fatigue is thought to induce a deterioration of executive control [179].

Barger and colleagues reported a mean sleep quantity of 6.09 hours on the ISS [15]. In our study, we showed that astronauts slept at least 5 hours the night before the recordings. Yet, we found that their sleep pressure was increased in space [114]. In a laboratory controlled condition, chronic sleep restriction (i.e. 5 hours of sleep opportunity per night) would be the close to what astronauts are experiencing.

Chronic sleep restriction is a simulation of an insufficient sleep syndrome. However, how chronic sleep restriction impacts behaviour is not fully understood [171]. Sleep restriction (i.e. 40 hours of wakefulness) strongly impact PVT scores, however, sleep restriction (i.e. 5 hours of sleep) has a milder effect on alertness [95, 171]. Nevertheless, chronic sleep restriction has shown to have local effect on slow wave activity in the prefrontal cortex during NREM sleep, which in turn push the subjects to take riskier decisions [171]. Insufficient sleep dissipation in the right frontal cortex would impact decision making but it could also impact psychological strain. In our second study we found, that an increase of theta power in the evening in the right frontal area was

associated with an increase of psychological strain[113]. Psychological strain has shown to impair working memory, task for which the prefrontal cortical regions are important [208].

5.1.2 Limitation of theta activity and local sleep-like events as sleep pressure markers

The gold standard measure of sleep pressure is SWA during sleep [30]. Even though theta activity and local sleep-like events during wakefulness are markers of sleep pressure, our results need to be carefully interpreted.

Sleep-wake history Our study was designed after data acquisition. Therefore, sleep-wake history was not carefully controlled in astronauts and we couldn't evaluate the circadian influence precisely. This limitation restrain the interpretation of our results [114]. Actigraphy data was recorded by the group of Dr. Dinges in two astronauts out of the five included in our study. A collaboration with Dr. Dinges would have been interesting, but wouldn't improve the interpretation of the results. To understand the effect of the circadian component on sleep pressure markers in space, we would need a single night sleep deprivation (i.e. 40 hours of wakefulness).

Noon and evening theta activity As introduced earlier in this work, the circadian component compensate the build up of the homeostatic pressure in the evening to maintain cognitive performances [106]. Accordingly, noon and evening theta activity don't have the same physiological implications. In further experiments it would be interesting to record theta activity at different time points within the same participants and study its relation to sleepiness and cognitive performances. So far, theta power was essentially studied after sleep restriction and its correlation with alertness scores is the strongest in the evenings [247, 95, 171]. We hypothesise that at noon with less circadian influence, theta power is more representative of the accumulated sleep pressure. Hence, noon could be the time of the day when countermeasures, such as physical activity, should be applied.

Theta power Theta activity increase in wake EEG has been related to performance decrease after sleep deprivation [47]. Local populations of neurons turning "off" in a network could be responsible for this decrease of performance after prolonged wakefulness. To confirm this hypothesis, we would need to further investigate whether theta power in wake EEG is linked to local sleep-like events after prolonged wakefulness. Human intracortical recordings in combination with surface EEG would be the only method to confirm the hypothesis that theta activity and local sleep-like events are representative of the same underlying events.

Depending on the time window in which the power spectrum is computed, a local increase of theta power is not always representative of an increase of local sleep-like events in the underlying cortical area. Theta power represents the combination of the amplitude and the occurrence of a certain type of events in a defined time window. If we assume that local sleep-like events appear in bursts of activity, the Fourier transform will most likely capture the events, However, if local sleep-like events are isolated events, the Fourier transform will not be sensitive enough to capture the events.

Local sleep-like events Using scalp EEG to study local sleep-like events is not similar to intracortical recordings. Even though Intracortical "off periods" recorded in rats are depicted as theta oscillations in intracranial local field potentials [253], local sleep-like events might be reflected differently at the scalp level. In SWS, the amount of neurons in a synchronous off state is correlated with the amplitude of the slow oscillations recorded at the scalp EEG [71]. During NREM sleep, SWA and alpha activity can be present simultaneously in different cortical areas, nevertheless SWS is the dominating oscillation [189]. During wakefulness, hyper-polarized neurons in an off state (i.e. local sleep-like event) could be intermingled with active neurons firing in the alpha range within the same cortical area [253]. Different oscillations within the same network will compete or interact with each other [43]. Following the linear superposition principle, two waves are interacting with each other while travelling in the same medium. Two sine waves with different frequencies will be recorded as a sine wave at the average frequency between the two and the amplitude will depend on the alignment of the two oscillations' phases. Hence, local sleep-like events at the scalp level might be recorded at a higher frequency than the 2-6 HZ intracortical recordings in rats. Furthermore, if "off periods" are only present in a small network, the amplitude of the signal at the scalp level might be too low to be detected by our algorithm. On the other hand, if a local sleep-like event appears more globally (i.e. neurons in an off state are spread on a larger area), the amplitude of the local sleep-like event should be increased. Nevertheless, in our study we found no difference in the amplitudes of the local sleep-like events, even though we found a difference in the number of electrodes involves in the events [114].

NREM sleep To confirm the interest of theta power and local sleep-like events as markers of sleep pressure, we would need interesting to record SWA the night before and after the experiment.

Theta activity in wake EEG In our analysis we cannot exclude that theta activity might be representative of other cognitive processes than sleepiness. Beside sleep pressure and sleepiness, theta activity during wakefulness has been associated with many cognitive processes.

In hippocampal place cells, bursts of activity occurs in the theta range. This modulation of hippocampal activity might be correlated with cortical activity [193]. Hippocampal theta activity has been associated with spatial navigation and recorded in rodents when screwing EEG leads in the skull [44] and in humans when recording intracranial signal in epileptic patients[149]. However, it is unclear if hippocampal theta oscillations can be recorded in humans by scalp EEG.

Interestingly, in the neocortex, gamma oscillations' amplitude is modulated in the theta range [51]. Theta modulation of these gamma oscillations has been associated with cognitive processes like speech processing [122]. Moreover, large scale neural network activity modulation on a theta frequency, especially fronto-parietal theta phase synchronisation, has shown to play a critical role in cognitive processes [181, 206].

5.1.3 Limitation of the ISS study

To the best of our knowledge, the Neurospat experiment recorded EEG data with the highest density ever recorded in space. Nevertheless, recording brain activity at the scalp level will not allow us to understand the underlying physiological changes occurring in space. To capture neuroplastic modifications with the time in space and assess anatomical changes correlation to behavioural observations, magnetic resonance imaging and diffusion tensor imaging studies would need to be performed, not only pre and post flight but also during the mission . Moreover, there is a need to foster investigations on short term adaption to the novel space environment, alongside with long term effect adaptation. Unfortunately, and due to the limits of the experimental protocol, we have only two recording sessions in space and we are unable to investigate if our findings are impacted by microgravity (short or long term effects) or if our findings are resulting from the sleep-wake history.

5.2 Long term isolation is not enough to mimic space implications on sleep pressure

Findings Studies conducted in Antarctica suggested that isolation, high altitude and constant darkness might disturb sleep in a similar fashion as on the ISS. Indeed, theses studies reported a sleep quality decrease and a misalignment of the sleep-wake cycles with the circadian clock during the winter in Antarctica [200]. In our study, unlike on the ISS, we found no differences in theta power during the isolation period at the Concordia station [113]. Nevertheless, our analysis revealed that high sleep pressure is linked to psychological strain in the evening and that physical activity at noon could be envisioned as a sleep pressure countermeasure.

5.2.1 Limitation of the Concordia study

Similar to the ISS study, without sleep-wake history it is impossible to assess if participants were misaligned, sleep restricted or even sleep deprived at the time of the recording. Most importantly, unlike the ISS study, we had no information about the night proceeding the recording, which limits the interpretation of our results. For one of the two years at Concordia (DC07), the group of Dr. Pattyn has conducted a sleep study. A collaboration with them would have improved considerably the value of our analysis for 5 participants (DC07) out of the 12 included in our study (DC07 & DC08). In our study, the correlation between theta power and psychological strain, needs to be further explored in a more controlled environment. We don't have access to the sleep-wake history before the recording and hence we cannot assume that participants showing an increase of theta power in the right frontal area were sleep restricted. Moreover, as mentioned in the previous section, noon and evening theta activity might have different physiological implications. The decrease of sleep pressure after physical activity at noon, and not in the evening, is only possible because of the circadian influence. However, the benefit for alertness and for total sleep debt of a decrease of sleep pressure at noon needs to be explored.

5.2.2 Other space environmental factors that might increase sleep pressure in space

Besides isolation, other psychological stressors like confinement and chronic stress can strongly disturb sleep [239]. During the MARS 500 analogue study, confinement has shown to increase sleep quantity in most participants, but confinement decreased sleep quality in some others and even provoked a free running behaviour in one participant [20]. Additionally, cortisol levels were associated to sleep disturbances during the same analogue mission [118]. Beside psychological factors, also physiological changes due to space environment can be responsible for an increase of sleep debt [239]. Humans have shown to acclimate to high altitude hypoxia [224]. Also, a fast acclimatisation was observed for the deregulation of the vestibular provoking space sickness [263]. However, acclimatisation might be more problematic for the fluid shift, induced by microgravity, and the immune system deregulation, induced by ionising radiations [263]. A human missions to Mars will be aggravating these phenomena and we need to disentangle the effect of all these factors before such a mission can be envisioned.

5.2.3 Other potential analogues for sleep studies

Analogue missions are not only an opportunity to study human acclimatisation to space-like environment, but they are also an unique opportunity to gain knowledge on populations like sedentary populations, clinical popula-

tions affected by insufficient sleep syndrome or chronic stress. Cardiovascular adaptation and osteoblast proliferation are also hot topics in space medicine. Bed rest studies are usually the leading analogue choice, since they mimic prolonged immobility and fluid shifts. Osteoporosis in elderly also studied for a long time in space analogues [49]. However, the mechanisms of bone density loss in osteoporosis on Earth might be different from the mechanisms bone loss in microgravity. Overall, all space countermeasures developed for space application can also be applied on Earth.

In the past two years I tried to run other experiments in space analogue environments. I contacted the dry immersion experiment in Toulouse (one month), the bed rest studies in Cologne (2 months), offered to record EEG in the Neutral buoyancy pool at the Astronaut center in Cologne, visited Hi-Seas in Hawaii (one year mission) and offered to record sleep in their next session. I talked with the Russian to take part in the next Sirius session (one year mission). Finally I choose to go with the European Space agency Astronaut training programme and we proposed an experiment for the Astronaut Caves training program (6 days 800m underground) [182], including portable EEG devices (Avatar devices from EGI systems) and a collaboration with Dr. Emily Coffey from the Jan Born lab in Tübingen. Unfortunately the 2018 session, for which we were selected was indefinitely delayed.

5.3 Potential sleep pressure countermeasures in space

For more than 20 years we know that good sleep pressure dissipation will be crucial for long term manned missions [93]. All activities on the ISS are aligned to the Coordinated Universal Time (UTC) and regulated working schedule with at least 8.5 hours of sleep opportunity were established as countermeasures. However, astronauts are still complaining about insufficient sleep in space [15]. We will enumerate the existing countermeasures and we will present some high readiness level countermeasures that could be implemented within a few years.

5.3.1 State of the art countermeasures

Light countermeasure Lessons were learned from Antarctica. Concordia station is in Casey Time Zone (CAST) which is the Coordinated Universal Time (UTC) with an offset of +8 hours in the winter and +11 hours in the summer. During the winter constant darkness period, it can be difficult to entrain the participants' circadian rhythm. Antarctica chronobiological experiments, reported circadian misalignment in the winter period due to a lack of light Zeitgeber. As a countermeasure, they successfully tested an artificial light therapy to shift the circadian rhythm and realign the sleep-wake cycle [37, 195]. On the ISS a similar countermeasure was implemented. Blue light

(460 nm) has shown to improve circadian clock shifting and improve alertness [48]. They are currently testing solid-state lighting, with blue light in its spectrum, to improve crew circadian alignment and cognitive performance [34].

Sleep promoting drugs The use of sleep promoting drugs (i.e. zolpidem) in 11% of the nights on the ISS has been reported [15, 19]. Sleep promoting drugs are commonly used but they are not desirable since they decrease the alertness of astronauts which be unable to perform in case of an emergency during the nigh.

Napping In the afternoon 20 min nap opportunity after lunch time has shown to be effective against sleepiness in space analogue missions [151]. Naps could help to counterbalance sleep debt, however the resulting sleep inertia, when waking up, do not benefit vigilance and alertness [132].

Psychomotor vigilance test "Cognition" is a battery of 10 tests (20-30min) developed by Prof. Dinges and NASA, to assess cognitive performances of astronauts on a regular basis during the mission and before an EVA [185]. Performances at the psychomotor vigilance test (PVT) has shown to decrease when sleep pressure is high [18]. The PVT is included in the "Cognition" battery of tests which prevent from sending astronauts on an extravehicular activity (EVA) when sleep restricted.

All the aforementioned state of the art countermeasures are already implemented for manned spaceflights, but they have shown to be insufficient. Urging the need for other sleep promoting methods.

5.3.2 Chronotype and vulnerability to sleep loss screening

For a good crew cohesion during a mission it is beneficial if the crewmembers have the same chronotype [124]. The chronotype is the interindividual differences in the endogenous circadian oscillation period. Besides the period, the amplitude of the circadian influence on sleep pressure can also vary between individuals. The consequences are that individuals preferences for sleep-wake timing is different (i.e. morning "lark" or evening "owl" persons) and adaptation to sleep loss might also vary. Chronotypes and sleep loss vulnerability can be defined by questionnaires and by genetic screenings [123]. Polymorphism in the circadian genes might be at the origin of such an interindividual difference. However, the identification of these genetic predictors is yet to be done [124].

5.3.3 Physical activity

long term effects Pattyn and colleagues in their Antarctica meta-analyse outlined the correlation between actigraphy data and sleep disturbances [200]. Daily physical exercise can help reduce sleep disturbances in Antarctica. However, interactions between sleep and physical exercises are not straight forward [60].

short term effects Physical activity designed as a learning task can induce use-dependent plastic changes which in turn locally increase SWA overnight [142]. In the other hand, an automatic physical exercise can reduce neuronal firing rate in some cortical areas and hence reduce the need for sleep recovery [108]. Local use-dependent regulations by means of specific physical exercises could reduce sleep debt in some cortical areas, when sleep quality and quantity is not sufficient. In our analysis of the Concordia experiment we looked at EEG recordings before and immediately after the physical exercise [113]. We saw a decrease of sleep pressure in most cortical areas besides the frontal areas, when the physical exercise was performed at noon. In our task an incremental bicycling task was performed. It is not surprising that neuronal activity in the motor and in the sensorimotor areas was impacted by the task. To study this compensation hypothesis, an interesting protocol would consist in chronic sleep restriction in a laboratory condition with an adapted physical exercise to compensate the lack of sleep recovery. Furthermore, to confirm the interest of physical activity as a countermeasure, further studies would need to record sleep EEG the following night to observe the SWA at the beginning of the night and study how SWA dissipates overnight.

5.3.4 Meditation

Since a proper control population is hard to find for experienced meditators, long term effects on the build up f sleep pressure is difficult to proof. Accordingly the decrease of sleep quantity need reported by Ferrarelli and colleagues[103] could be caused by other factors than meditation per se. Nevertheless, to follow this line of research, it would be interesting to explore the short term effects of meditation on local sleep pressure in new meditators. Similar to physical activity, meditation in non experimented meditators could induce use-dependent plastic changes that will be mirrored during SWA.

Besides sleep improvement, long term effects of meditation and also short term (i.e. few weeks) effects of mindfulness meditation have shown to improve alertness [236]. Moreover, when think of the design of a transplanetary space mission, every kilogram of the payload is critical for the mission design. Daily meditation could decrease the respiration rate which in turn will decrease the metabolism and reduce the need in supply for a manned crew [136, 235].

5.3.5 Adenosine antagonist

The concentration of the neuromodulator adenosine builds up with the time awake and is representative of the neuronal activity history. Adenosine levels fall during SWS. In the basal forebrain, adenosine acts as a neuromodulator for the cholinergic neurons and in turn inhibit the wake-promoting neuronal circuits [17].

Caffeine is a common adenosine antagonist and is known to prolong wakefulness and increase attention when sleep pressure is high. Caffeine reduce subjective sleepiness and theta activity during sleep deprived wakefulness for 1-4 hours [158]. One hypothesis is that caffeine could slow down the build up of sleep pressure with the time awake.

The role of adenosine in sleep homeostasis is not fully understood yet and caffeine sensitivity varies between individuals [211]. Even though caffeine could be an interesting countermeasure to decrease sleep pressure and increase alertness, its intake increase sleep latency, decrease SWA the following night and tend to shift the circadian rhythm [67].

5.3.6 Acoustic stimulation during NREM sleep

Another solution to reduce sleep pressure could be a non–invasive sleep modulation tool which is able to enhance sleep quality and which will maintain astronauts' cognitive performances in space over time. The Close loop stimulation tool takes as an input the signal of a single EEG channel and detects in real time the brain oscillations of interest. As an output and time locked to the signal of interest, a stimulus is delivered (e.g. sound). Depending on the phase of stimulation it has shown to entrain or reduce the amplitude of slow oscillations during NREM sleep [101, 186]. By locally manipulating slow wave oscillations, we can locally increase SWA and improve cognitive performances associated with this cortical area [101, 262]. As an alternative to sleep stimulation, we could think of similar simulations during wakefulness to reduce sleep pressure, while targeting theta oscillations at noon when the circadian influence is at its minimum. To the best of our knowledge the long term effects of the close loop stimulation tool has never been assessed so far.

5.4 Other countermeasures and future perspectives

Beside improving sleep other space countermeasures are needed for risk mitigation during long term space travel [239, 263].

5.4.1 Artificial gravity

A human centrifuge would be a straight forward countermeasure to sustain an artificial loads at 1g [112]. Artificial gravity is the only valuable counter-

measure to simulate weight bearing in microgravity. A onboard centrifuge would also allow an early adaptation to the 0.166g on the Moon and the 0.38g on Mars. As an intermediate step, a physical exercise performed on the ISS could be conducted under artificial gravity. So far, a bicycle ergometry exercise combined with a 1.4m radius centrifuge has been designed for an ISS module [88]. A Japanese bed rest study conducted such a ground based study, using a bicycle ergometry exercise in artificial gravity as a countermeasure for the cardiovascular system [146]. In Europe, the Envihab facility in Cologne has a ground based short-arm human centrifuge and analogue studies are currently conducted during bed rest studies. Astronauts are carrying out two hours of physical activity (resistance exercises, bicycle, treadmill [164]) per day. Daily physical activity has shown to slow down the bone density loss but it can't counteract the total loss of bone density [159]. Artificial gravity is promising countermeasure for bone and muscle atrophy.

5.4.2 Magnetic shielding

Because of payload mass constraints for mission feasibility, shielding of the spacecraft is very difficult in space [99]. Moreover, current shielding technologies don't protect against secondary particles (i.e. aluminium 10 g/cm2) [268]. One alternative solution would be to use water as a shielding material (6.3 g/cm2) [156] or induce an electromagnetic field around the transfer habitat. To maintain an electrostatic shield, we would need at least a 20 T magnetic field to create an high density plasma around the spacecraft able to deflect comic rays [197]. The readiness level of superconductors for this technological application remain unfortunately in the science fiction realm.

5.4.3 Hibernation

Induction of an hibernation-like state in astronauts would be a game changer for human space exploration [63, 203]. For a manned mission to Mars, the reduction of the metabolism could considerably reduce the need of consumables (i.e. 10 tonnes) [203]. Besides reducing the payload, the reduction of movements triggered by the hibernation-like state would allow a transit habitat design with a smaller volume. A prolonged hibernation state would reduce social interactions during the transit between two planet's orbits. As a consequence, the psychological stress of prolonged isolation would be reduced. Hibernation also prevent the degradation of muscles and bones when disused [72]. The induction of a cellular quiescence state and the adaptation to hypoxia during hibernation can protect tissues against cold, against ischemia and against radiations [180, 58, 125]. Instead of heavy shielding, hibernation could be an alternative solution against ionising radiations. We need to understand and develop safe mechanisms to counteract long term effects of microgravity and enhance resistance to radiations and hibernation could be an alternative

solution for most of the countermeasures mentioned in the previous section. The protective aspects of hibernation are multiple and could keep astronauts mentally and physically healthy during their journey [259].

Current hypothermia clinical therapies lower the temperature of patients and consequently induce a reduction of the metabolism. To achieve this the therapies use pharmacological thermoregulation inhibitors and external cooling devices. Opposite to these therapies, hibernation is an endogenous metabolic reduction which may or may not induce a reduction of the core body temperature, depending on the environment temperature [134]. For an human hibernation-like state, we would prefer to keep a high core body temperature, as observed in large mammals (i.e. bears). Higher temperature (¿30 °C) would limit the need for inter torpor bouts arousals and would allow a faster termination of hibernation if needed [79]. First, extensive research need to be conducted on animal models that usually don't hibernate (e.g. rats and pigs) [63].

5.4.4 Animal studies

In Earth analogue conditions we can mimic isolation, limited physical activity, chronic stress load, artificial body fluid shift. However, space is the only setup where Earth gravitational field can be removed. Moreover, the impact of ionising radiations is still poorly understood. Besides hibernation research, a lot of clinical pathologies (e.g. osteoporosis, cardiovascular disorders, oncology) could benefit from research in space [259]. Research on animal models in microgravity is not used to its full extend. We should take advantage of animal facilities on the ISS to send animals to space. ISS platforms for rodents research, like the Animal Enclosure Module and the Mice Drawer System, function autonomously for more than 3 months and need to involve astronauts only for maintenance activities [50]. Animal models should be employed to understand the effects of microgravity and radiations that cannot be modelled in human analogue studies.

5.5 Conclusion

After 20 years of activity, the ISS programme was supposed to end in 2020. Two years ago the international community decided to prolong the funding until 2024. However, the Trump administration might end the programme earlier. Future human physiology experiments in space would need to be conducted in collaboration with China. China space station will be open to all UN nations for scientific experimentation, starting 2022. Sleep research should be first in line. Taken together, our results suggest that sleep deficiency, if not monitored, might be a hurdle for further space mission. However, more experiments in a microgravity environment would be needed to confirm our

first evidence of sleep pressure increase in space. Current countermeasures leave space for improvement. We proposed a list of countermeasures that would need to be explored to enable manned exploration beyond Earth orbit.

Chapter 6

List of publications

ISS paper

Local sleep-like events during wakefulness and their relationship to decrease in alertness in five astronauts on the International Space Station. Petit G, Cebolla AM, Fattinger S, Petieau M, Summerer L, Cheron G and Huber R. *Accepted with NPJ Microgravity* (January 2019).

Concordia paper

Electrophysiological measures of sleep pressure during wakefulness in the course of isolation at the Concordia Antarctica station and physical activity as a countermeasure. Petit G, Abeln V, Summerer L, Schneider S and Huber R. *bioRxiv* (January 2019).
https://www.biorxiv.org/content/early/2019/01/09/516567

Hibernation chapter

Hibernation and Torpor: Prospects for Human Spaceflight. Petit G, Koller D, Summerer L, Heldmaier G, Vyazovskiy VV, Cerri M & Henning RH. *Springer*, Handbook of Life Support Systems for Spacecraft and Extraterrestrial Habitats:1–15, (September 2018).
https://link.springer.com/referenceworkentry/10.1007

Chapter 7

Acknowledgement

I want to thank my main supervisor and the principal investigator of my project, Prof. Reto Huber. Thank you for your trust and for your constant support during these three last years. Thank you for letting me collaborate with ESA and for allowing me to conduct my own projects. Without your continuous feedback and advise, I would not have make it. Also a big thank to Prof. Reto Huber's lab at the Children Hospital for their friendship and for their critical mind when it comes to discussing results. So thank you to Dr. Anne-Laure Mouthon, Dr. Salome Kurth, Flavia Wehrle, Carina Volk, Natalie Heyse, Valeria Jaramillo, Georgia Sousouri, Joelle Albrecht, Melanie Furrer, Niklas Schneider, Michelle Suppiger, Dr. Sara Fattinger, Elena Krugliakova, Mirjam Studler, Sophia Snipes and Dr. Angelina Maric for being amazing co-workers.

I want to thank Dr. Leopold Summerer for hosting me at the European Space Agency's Advanced Concepts Team (ACT). Thank you for supporting my ideas, for using your network to make the projects happen and for your help in setting the Ariadna grant and defining the MuOs. Thank you to Dario Izzo for your great scientific mind and for critical remarks. Thank your to Dominik Koller for being a great neuroscientist colleague at ESA and for helping me in most of my projects. Thank you to all my colleagues and friends form the ACT, you made my time at ESA unforgettable. So thank you to Rohan Ramasamy, Dharmesh Tailor, Dr. Jay Grover, Andrea Guarriello, Alessio Mereta, Andrej Krzic, Alexander Hickey, Marlies Arnhof, Hanna Lakk, Dr. Thomas Vasileiou, Dr. Marcus Märtens, Dr. Alexandre Feller, Dr. Benjamin Rouselle and Martin Nisser for being amazing co-workers.

I want to thank my collaborators form the Université Libre de Bruxelles (ULB), for their enthusiasm and for the most interesting collaboration. Thanks to your help we achieved more than I imagined. I hope we will be able to collaborate again in the future. So tank you to Dr. Anita Cebolla, Prof. Guy Cheron and Mathieu Petieau.

I want to thank my collaborators from the Deutsche Sporthochschule Köln, for sharing with us their most interesting dataset and for being supportive along the process. So thank you to Dr. Vera Abeln and Prof. Stefan Schneider.

Thank you to the ESA topical team on Hibernation. Thank you for inviting me in your consortium and for collaborating with me. A special thank to my co-authors, I learned a lot from our discussions and I hope we will keep working together in the future. Thank you to Prof. Gerald Heldmaier, Prof. Vlad Vyazovskiy , Prof. Matteo Cerri, Prof. Rob Henning and Prof Alexander Chouker.

I want to thank Prof. Christian Baumann, for first interviewing me when I was still doing my master thesis in Boston and for always supporting me in this thesis.

I want to thank Prof. Nicole Wenderoth, for your interest in my project and for supporting me with your feedback these past years.

Finally thank you to Rafael Polania for being the chair of my doctoral examination.

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